Nature Reviews Rheumatology | Published online 6 Jul 2017; doi:10.1038/nrrheum.2017.109

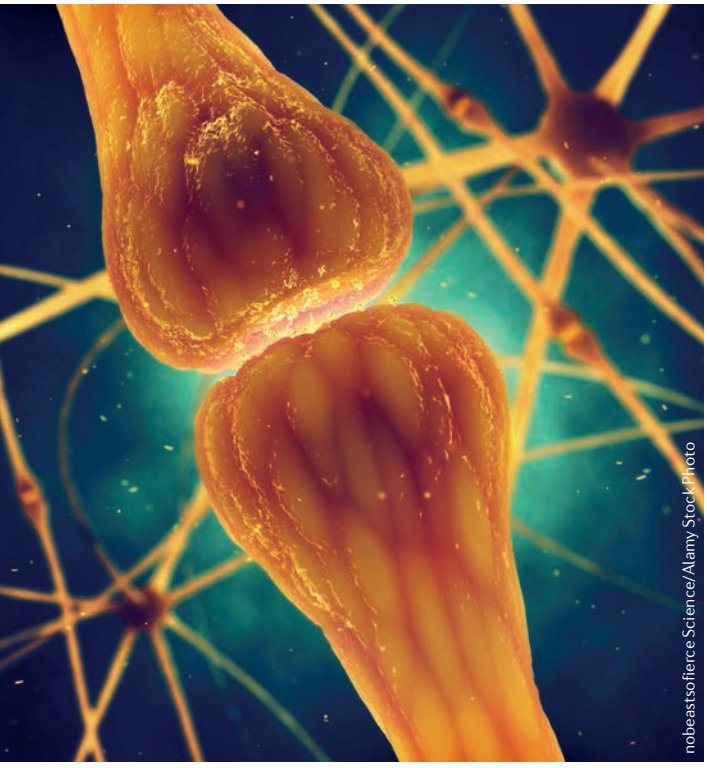
SYSTEMIC LUPUS ERYTHEMATOSUS

IFN drives synapse loss via microglia

Microglia are critical mediators of central nervous system (CNS) disease manifestations in systemic lupus erythematosus (SLE), according to new findings published in *Nature*. "In the field of SLE research, the mechanisms of CNS disease in SLE have been unclear," explains corresponding author Michael Carroll. "We identify type I interferon (IFN) as a modulator of microglia function that can stimulate synapse loss and microglia engulfment of synaptic material."

"While work by Betty Diamond and others have shown that autoantibodies can contribute to CNS lupus, these instances do not fully account for the high prevalence and variety of neuropsychiatric symptoms in patients with SLE," states Allison Bialas, first author of the paper.

> "...lupus-prone mice had increased numbers of reactive microglia compared with their wild-type littermates, which could be reduced with anti-IFNAR treatment"



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The investigators characterized the behaviour of lupus-prone mice, finding that the behavioural phenotypes indicative of CNS disease could be blocked with administration of a type I interferon receptor (IFNAR)-blocking antibody.

The lupus-prone mice had no signs of inflammation or cellular infiltration of the brain, suggesting the involvement of CNS-resident cells rather than infiltrating immune cells in CNS manifestations. Furthermore, quantitative PCR analysis revealed increased expression of *Ifna* and the IFN-stimulated gene *Mx1* in the spleen of these mice, whereas in the brain only *Mx1* expression was increased, indicating upregulation of IFNAR signalling in the brain by systemic type I IFN.

The most prevalent *Mx1*-positive cell type was microglia (resident macrophages of the brain). A higher proportion of *Mx1*-positive microglia had a reactive phenotype than *Mx1*-negative microglia and indeed lupus-prone mice had increased numbers of reactive microglia compared with their wild-type littermates, which could be reduced with anti-IFNAR treatment. Using bone marrow chimeras to separate the effects of peripheral inflammation, Bialas *et al.* found that the increased number of reactive microglia was dependent on IFNAR, but not on IFNAR expression by peripheral immune cells, supporting a direct effect of IFNAR on microglia.

Synapse density in the frontal cortex was lower in lupus-prone mice relative to wild-type mice; this synapse loss was reduced with anti-IFNAR treatment. To investigate the mechanism, lupus-prone mice were crossed to mice expressing a GFP-fusion protein that localizes throughout neurons, enabling visualization of neuronal material uptake. Using this model, microglia of lupus-prone mice were demonstrated to internalize more neuronal material than those of wild-type controls, a finding also confirmed by transmission electron microscopy imaging. This engulfment correlated with *Mx1* expression, and was decreased with anti-IFNAR treatment and stimulated with biotinylated IFNα or IFNβ treatment.

"Going forward, we are working to understand whether changes in neurons stimulate microglia to target synapses or whether microglia are truly initiating this process," says Carroll. "We are also investigating whether synapse loss in lupus-prone mice is complement-dependent or whether other phagocytic pathways have a role and, finally, how this mechanism might fit in with antibody-mediated damage to the CNS."

"This study offers new insights into neuropsychiatric lupus," states Betty Diamond, who was not involved in the study. "It will be important to pursue these observations to learn if IFN also has direct toxic effects on neurons in lupus models and whether IFN-blockade leads to a reversal, not just a prevention, of behavioural phenotypes".

Although phase III clinical trials of anti-IFNAR therapies are currently underway, Bialas points out that these trials do not include patients with CNS symptoms or indeed include such symptoms in their clinical end points. "We hope that our work can be useful in designing future trials focused on CNS lupus," Bialas concludes.

Jessica McHugh

ORIGINAL ARTICLE Bialas, A.*et al.* Microglia-dependent synapse loss in type I interferon-mediated lupus. *Nature* http://dx.doi.org/10.1038/nature22821 (2017)

Nature Reviews Rheumatology | Published online 29 Jun 2017

IN BRIEF

SYSTEMIC LUPUS ERYTHEMATOSUS

Tackling complexity through immunophenotyping

Researchers have used an immunophenotyping approach to categorize patients with systemic lupus erythematosus (SLE) into distinct subgroups. Peripheral blood mononuclear cells from 143 patients with SLE and 49 healthy individuals were analyzed by flow cytometry to characterize circulating B cells, T cells and dendritic cells. The resulting immunophenotype was analyzed by use of principal component analysis, and cluster analysis subsequently revealed three distinct subgroups based on T cell heterogeneity, including a T cell-independent group, a T follicular helper (T_{FH}) cell-dominant group and a regulatory T cell-dominant group. The percentage of patients with SLE who were resistant to immunosuppressive treatment was highest among the T_{FH} cell-dominant group.

ORIGINAL ARTICLE Kubo, S. *et al.* Peripheral immunophenotyping identifies three subgroups based on T cell heterogeneity in lupus patients. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40180 (2017)

RHEUMATOID ARTHRITIS

ACPA status influences RA development

A longitudinal study has identified differences in the clinical manifestations of patients with anticitrullinated protein antibody (ACPA)-positive ($n = 30$) and ACPA-negative ($n = 37$) rheumatoid arthritis (RA) during the pre-RA phase. Initial symptoms involved the lower extremities more often in the ACPA-positive group. At first presentation with arthralgia, ACPA-positive patients had a longer symptom duration, lower number of tender joints and less difficulty making a fist. However, ACPA-positive patients developed arthritis sooner after presenting with arthralgia than ACPA-positive patients.

ORIGINAL ARTICLE Burgers, L. E. *et al.* Differences in the symptomatic phase preceding ACPA-positive and ACPA-negative RA: a longitudinal study in arthralgia during progression to clinical arthritis. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211325 (2017)

CRYSTAL ARTHRITIS

Combination therapy effective in tophaceous gout

The phase 3 CRYSTAL trial investigated the efficacy of combining lesinurad (200 mg or 400 mg), a selective urate transporter inhibitor, with febuxostat treatment for tophaceous gout. The proportion of patients achieving serum urate levels <5.0 mg/dl at 6 months (the primary end point) was higher among patients receiving 400 mg lesinurad in addition to 80 mg febuxostat than among patients receiving febuxostat alone. At all other time points (up to 12 months), 200 mg lesinurad plus febuxostat was more effective than 400 mg lesinurad plus febuxostat in achieving the target levels of serum urate.

ORIGINAL ARTICLE Dalbeth, N. *et al.* Lesinurad, a selective uric acid reabsorption inhibitor, in combination with febuxostat in patients with tophaceous gout: a phase III clinical trial. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40159 (2017)

THERAPY

Rheumatic disease after immune checkpoint inhibitor therapy

A retrospective analysis of a French registry including patients with cancer treated with immune checkpoint inhibitors revealed six cases of rheumatoid arthritis (RA) and four cases of polymyalgia rheumatica (PMR), which developed at a median of 1 month after exposure. Three patients who developed RA required DMARD therapy; the other three cases were treated with corticosteroids or NSAIDs. All four patients with PMR responded to corticosteroids.

ORIGINAL ARTICLE Belkhir, R. *et al.* Rheumatoid arthritis and polymyalgia rheumatica occurring after immune checkpoint inhibitor treatment. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2017-211216 (2017)

Nature Reviews Rheumatology | Published online 29 Jun 2017; doi:10.1038/nrrheum.2017.108

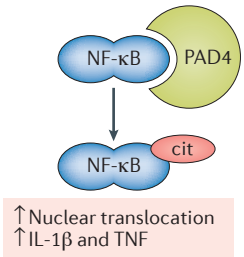
RHEUMATOID ARTHRITIS

Growing role for PADs in pathogenesis

> A haplotype of *PADI4*, which encodes PAD4, is a known risk factor for RA

Peptidylarginine deiminases (PADs) are responsible for the citrullination of many proteins, a posttranslational modification known to be important in the pathogenesis of rheumatoid arthritis (RA). Citrullination of histones is a crucial step in the formation of neutrophil extracellular traps (NETs), but a new study has extended the role of PADs beyond NET formation, or NETosis, to the production of pro-inflammatory cytokines.

Researchers Bo Sun and Nishant Dwivedi, together with their colleagues, characterized a novel relationship between PAD4 and the transcription factor NF-κB. Citrullination of the p65 subunit of NF-κB by PAD4 increased the binding of this subunit to importin α3 in neutrophils, thereby increasing the rate of nuclear translocation of NF-κB and the subsequent production of the pro-inflammatory cytokines IL-1β and TNF. "Our data set an example of how citrullination can directly modulate the function of transcription factors and immune cells," says I-Cheng Ho, corresponding author on the study. "By promoting the expression of inflammatory cytokines, such as TNF and IL-1β, hypercitrullination can also actively contribute to the pathogenesis of RA," he continues.



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A haplotype of *PADI4*, which encodes PAD4, is a known risk factor for RA; this risk haplotype contains three missense mutations located away from the site of enzymatic activity. The researchers showed that these missense mutations increased the affinity of the resulting PAD4 variant for NF-κB p65 compared with normal PAD4. This increased affinity led to enhanced citrullination, nuclear translocation and expression of pro-inflammatory cytokines *in vitro*, providing a possible explanation for the increased risk of RA in carriers of this gene variant.

Using mass spectrometry and computer modelling, the researchers identified four arginine residues on NF-κB p65 that they believe are crucial for the increased activity observed after citrullination by PAD4. "In the near future, we plan to characterize in detail the dynamic interaction between PAD4 and NF-κB p65 and understand how citrullination of p65 enhances its interaction with importins," reports Ho.

"The paper opens the door for many interesting new studies, both on basic mechanisms and on therapies using various PAD inhibitors," states Lars Klareskog, who was not involved in this study. "Specifically targeting the interaction between PAD4 and NF-κB p65 may allow us to inhibit the activity of NF-κB without the unwanted effects of global inhibition of PAD activity," explains Ho.

"The role of citrullination in neutrophil activation was already known and believed to be of importance in RA, in particular in relation to the influence of ACPAs on NETosis, and the present findings add significantly to the understanding of the potential role of neutrophils in RA and other inflammatory diseases," says Klareskog. "In addition, the findings identify a new and interesting and cell specific potential target for therapy," he concludes.

Joanna Collison

ORIGINAL ARTICLE Sun, B. *et al.* Citrullination of NF-κB p65 promotes its nuclear localization and TLR-induced expression of IL-1β and TNFα. *Sci. Immunol.* **2**, eaal3062 (2017)

Nature Reviews Rheumatology | Published online 8 Jun 2017; doi:10.1038/nrrheum.2017.97

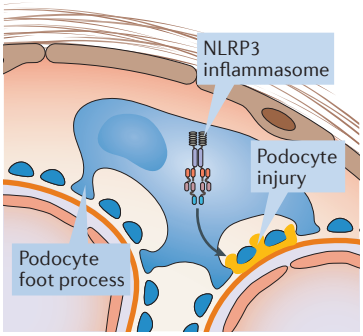
LUPUS NEPHRITIS

NLRP3 inflammasome ignites podocyte dysfunction

> the NLRP3 inflammasome was found to be activated in podocytes from biopsy and urine samples from patients with lupus nephritis

Activation of the NLRP3 inflammasome results in podocyte injury and proteinuria in lupus nephritis, according to a new study. "The most significant finding in this project is that podocytes are active participants in the pathogenesis of lupus nephritis," says Niansheng Yang, corresponding author of the study.

Podocytes are highly specialized cells surrounding the glomerular capillaries and have a major role in blood filtration in the kidneys. Podocyte dysfunction is known to be involved in the pathogenesis of lupus nephritis, but the molecular mechanisms underlying the injury of these cells have not yet been elucidated. In this study, Yang and colleagues investigated the role of the NLRP3 inflammasome, a molecular complex of the innate immune system that activates caspase 1 and leads to the production of pro-inflammatory cytokines such as IL-1β.



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The investigators showed that the protein expression levels of NLRP3 and IL-1β were higher in NZM2328 lupus-prone mice with severe proteinuria than in control NZM2328 mice without proteinuria. Furthermore, the levels of active caspase 1 in podocytes from mice with proteinuria were higher than in those from control mice. Consistent with these findings, the NLRP3 inflammasome was found to be activated in podocytes from biopsy and urine samples from patients with lupus nephritis, but not in those from healthy donors.

To further confirm the role of NLRP3 *in vivo*, Yang and colleagues treated NZM2328 mice with the selective NLRP3 inhibitor MCC950. Treatment with MCC950 significantly reduced the levels of IL-1β in the kidneys and inhibited the activation of caspase 1 in glomerular podocytes, as compared with mice treated with vehicle. In addition, treatment with MCC950 reduced the incidence of severe proteinuria in NZM2328 mice compared with vehicle-treated mice, which was associated with an attenuation of podocyte foot process effacement and a reduction of renal lesions.

These findings indicate that the NLRP3 inflammasome is activated in podocytes and contributes to the pathogenesis of lupus nephritis. Yang remarks that their basic findings provide impetus for further research on the interaction between podocytes and inflammatory cells, which might help to identify novel targets for therapy.

Dario Ummarino

ORIGINAL ARTICLE Fu, R. *et al.* Podocyte activation of NLRP3 inflammasomes contributes to the development of proteinuria in lupus nephritis. *Arthritis Rheumatol* http://dx.doi.org/10.1002/art.40155 (2017)

Nature Reviews Rheumatology | Published online 29 Jun 2017; doi:10.1038/nrrheum.2017.91

SPONDYLOARTHROPATHIES

MIF drives inflammation and bone formation in AS

> MIF induced monocyte-specific TNF production and enhanced mineralization ... in osteoblast cell lines

Inflammation and new bone formation are hallmarks of disease progression in ankylosing spondylitis (AS). A new study published in *Arthritis & Rheumatology* now shows that macrophage migration inhibitory factor (MIF) not only promotes inflammation, but also triggers osteoblastic activity, suggesting a novel pathogenic role for this pleiotropic cytokine in AS.

The study's corresponding author Nigil Haroon explains that his group's interest in this area started with reports of antibodies against CD74 being detected in patients with spondyloarthritis. Given that CD74 is the cognate receptor of a potent pro-inflammatory cytokine (MIF), and that MIF reportedly also had effects on bone, their current work tested the hypothesis that the MIF–CD74 axis could represent a link between inflammation and new bone formation in AS. "We have shown that MIF can drive both processes effectively and its effects seem to be mediated through its receptor CD74," explains Haroon.

The investigators first demonstrated that serum levels of MIF were raised in patients with AS as compared with healthy individuals. Moreover, serum MIF levels were higher in patients with AS who were defined as 'progressors' (that is, those with a rate of increase in their modified Stoke AS spine score (mASSS) of ≥1 unit per year) than in 'non-progressors', and both serum MIF level and baseline mASSS independently predicted radiographic progression.

Tissue analyses revealed increased levels of MIF in the synovial fluid and high frequencies of MIF-producing macrophages and Paneth cells in the ileum of patients with AS.

In vitro, MIF induced monocyte-specific TNF production and enhanced mineralization and osteoblastic gene expression in osteoblast cell lines. MIF signalling was found to act via activation of β-catenin.

The researchers plan to explore MIF as a biomarker for AS diagnosis and prognostication. "We are also exploring therapeutically targeting MIF in AS," says Haroon.

Sarah Onuora

ORIGINAL ARTICLE Ranganathan, V. *et al.* Macrophage migration inhibitory factor induces inflammation and predicts spinal progression in ankylosing spondylitis. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40175 (2017)
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Nature Reviews Rheumatology | Published online 22 Jun 2017; doi:10.1038/nrrheum.2017.101

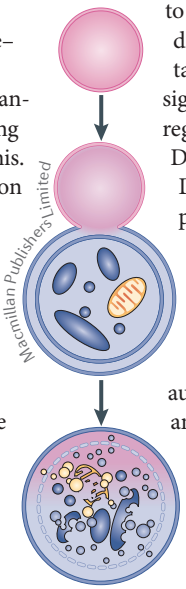
IMMUNOLOGY

Inhibiting autophagy in dendritic cells

> “Down-modulation of autophagy in [dendritic cells] compromised their ability to present autoantigen, prime T cells and induce experimental autoimmune encephalomyelitis.”

New findings have shed light on the molecular mechanisms by which regulatory T (T_{reg}) cells suppress dendritic cells (DCs), thereby suppressing autoimmunity. “T_{reg} cells potently suppress autoimmune responses *in vivo* through inhibition of the autophagic machinery in dendritic cells in a cytotoxic T-lymphocyte–associated protein 4 (CTLA4)-dependent manner,” states corresponding author Panayotis Verginis.

“Aberrant T_{reg} function has been closely linked to the development of autoimmune diseases and T_{reg} cell-based therapies hold great promise,” explains Verginis. “The clinical implementation of these therapies however has been hampered by a lack of understanding on the mechanism of their action.”



Verginis and colleagues sought to investigate the molecular targets underlying T_{reg} cell-mediated modulation of DC function, demonstrating in mice that autoantigen-specific T_{reg} cells inhibited induction of autophagy and autophagolysosome formation in DCs. Exposure of DCs to autoantigen-specific T_{reg} cells downregulated the mechanistic target of rapamycin (mTOR) signalling pathway, a well-known regulatory pathway in autophagy. Downmodulation of autophagy in DCs compromised their ability to present autoantigen, prime T cells and induce experimental autoimmune encephalomyelitis.

In the presence of a CTLA4-blocking antibody, T_{reg} cells lost their ability to downregulate autophagy in DCs, leading Verginis and colleagues to investigate the effects of abatacept (a CTLA4-Ig fusion protein) on murine bone-marrow-derived DCs. “Mechanistically, CTLA4 binding promoted activation of the mTOR pathway and Forkhead box protein O1 (FOXO1) nuclear exclusion in dendritic cells, leading to decreased transcription of the autophagy components,” says Verginis. DCs derived from patients with rheumatoid arthritis being treated with abatacept also displayed diminished autophagy compared with those derived from patients with rheumatoid arthritis undergoing anti-TNF therapy.

“These findings are clinically relevant and of potential therapeutic use,” remarks Verginis. Looking to the future, his group aim to focus on innovative methods to efficiently target autophagy in DCs. “Such an approach could pave the way for the development of new therapies not only in the field of autoimmunity but also in transplantation,” Verginis concludes.

Jessica McHugh

ORIGINAL ARTICLE(S) Alissafi, T. *et al.* Tregs restrain dendritic cell autophagy to ameliorate autoimmunity. *J. Clin. Invest.* http://dx.doi.org/10.1172/JCI92079 (2017)

Nature Reviews Rheumatology | Published online 8 Jun 2017; doi:10.1038/nrrheum.2017.93

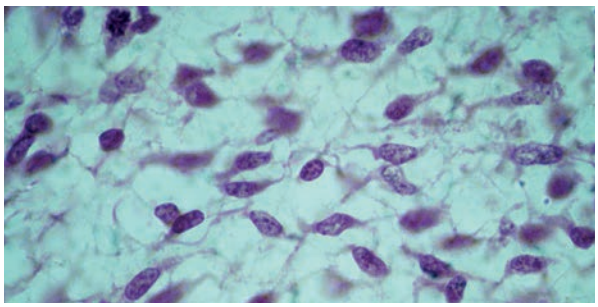
STEM CELLS

Synovial stem cells respond to a YAP

> GDF5-lineage [mesenchymal stem cells] were located in the synovial lining and in perivascular locations

Growth/differentiation factor 5 (GDF5)-lineage mesenchymal stem cells (MSCs) residing in the synovium of adult mice respond to cartilage injury and aid repair via the actions of transcriptional co-activator YAP1, according to new research. "Our findings show an important role for adult GDF5-lineage MSCs in the response to joint injury and provide a scientific rationale for the clinical use of MSCs from synovium for joint surface regeneration," states Cosimo De Bari, corresponding author of the study.

GDF5 is expressed in cells of the embryonic joint interzone, which form the synovial joint tissues during development. "We sought to identify GDF5-lineage cells in adult synovium and determine whether this cell lineage acts as a postnatal joint stem/progenitor cell reservoir," explains De Bari. To do this, the researchers crossed *Gdf5*-Cre mice (in which Cre recombinase is expressed in the joint interzone during development) with TdTomato reporter mice, which enabled them to trace cells of the GDF5 lineage in adult mice.



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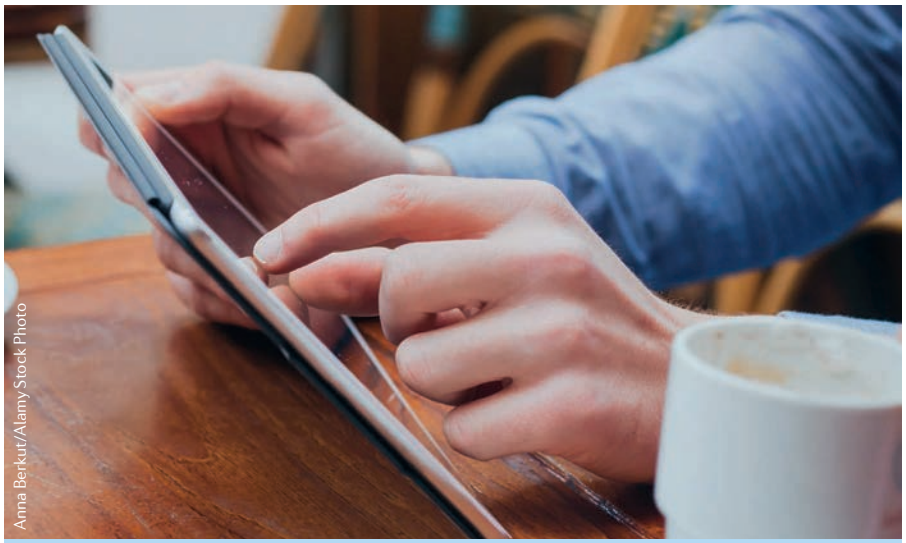
GDF5-lineage cells persisted in the synovium of adult mice, where they formed a distinct population that did not overlap with populations of other synovial cells or previously identified stem cells. These GDF5-lineage MSCs were located in the synovial lining and in perivascular locations. *In vitro*, these cells generated cartilage and synovial-like tissue, and *in vivo*, were able to repair cartilage injury when injected into cartilage lesions.

In mice with injured cartilage and in patients with traumatic joint injuries or osteoarthritis, the resultant hyperplastic synovium showed increased levels of YAP1. Furthermore, GDF5-lineage synovial MSCs were expanded following cartilage injury in mice and contributed to repairing the injury. "In the absence of YAP1, the GDF5-lineage MSCs in mice failed to drive synovial lining hyperplasia after injury, and showed a decreased recruitment to the cartilage repair tissue, implicating YAP1 as a key driver of the MSC response to injury," says De Bari.

Further research is required to fully understand the mechanisms behind the ability of these cells to respond to cartilage injury. "Understanding the underpinning mechanisms would open up new exciting avenues for the development of therapies to repair damaged joint tissues and prevent osteoarthritis," enthuses De Bari.

Joanna Collison

ORIGINAL ARTICLE Roelofs, A. J. *et al.* Joint morphogenetic cells in the adult mammalian synovium. *Nat. Commun.* **8**, 15040 (2017)



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SYSTEMIC LUPUS ERYTHEMATOSUS

The promise of PROMIS — is it ready for prime time in SLE?

Meenakshi Jolly and Patricia Katz

Patient-reported outcomes are important predictors of morbidity and mortality. The validity and reliability of the Patient Reported Outcomes Measurement Information System (PROMIS) computerized adaptive tests (CATs) has been assessed in patients with systemic lupus erythematosus, but does PROMIS deliver on its promise?

Refers to Kasturi, S. *et al.* Validity and reliability of Patient Reported Outcomes Measurement Information System computerized adaptive tests in systemic lupus erythematosus. *J. Rheumatol.* http://dx.doi.org/10.3899/jrheum.161202 (2017)

Improvements in the survival rates of patients with systemic lupus erythematosus (SLE) have shifted the focus of health care providers towards decreasing morbidity and improving quality of life (QOL). Patient-reported outcomes (PROs) provide unique health information that is best known and reported by the patient and are critical for assessing QOL. Evaluating PROs enhances our understanding of issues relevant to patients and facilitates a better understanding of patients' priorities and health concerns. PROs are also important components of clinical trials, are necessary for analysing cost-effectiveness, health disparities and the efficiency of health systems, and PRO information is increasingly requested by those who reimburse health care costs. New research by Kasturi *et al.*[1] has examined the use of computerized adaptive tests (CATs) for PROs in patients with SLE. CAT questionnaire methodology promises the use of fewer, more informative items, enabling more precise measurement and less burdensome surveys.

Given the prevalence of SLE among young people and women, and its multi-systemic involvement, unpredictable flares and disease chronicity, the effects of SLE (or its treatment) might be apparent in multitude of QOL domains. In addition to physical and emotional function, role performance, fatigue, pain, sleep, cognition, body image, adverse events from medication and social support are some of the domains identified by patients with SLE as pertinent to QOL[2,3].

PROs can be measured using generic or disease-specific tools. For example, the 36-Item Short-Form Health Survey (SF-36) has been validated for use in SLE and is the most commonly used generic tool in this disease; however, the SF-36 lacks domains that are particularly relevant to SLE, does not seem to be sensitive to changes in SLE disease activity and lacks SLE-specific minimally clinically important differences. The Patient Reported Outcomes Measurement Information System (PROMIS) initiative was undertaken to improve and standardize the measurement of PROs. PROMIS is a generic PRO system that enables comparison across disease groups and with the general population. The development of PROMIS utilized item-response theory to construct item banks; PROMIS domains can be selected to suit a specific health condition, with questions related to selected domains administered via CATs or as static short-forms.

Although short-forms of PROMIS have been evaluated cross-sectionally in patients with SLE[4], Kasturi *et al.*[1] are the first to evaluate the measurement properties of PROMIS CATs in outpatients with SLE ($n = 204$). The authors selected 14 PROMIS domains based on previous qualitative SLE focus group studies used for the development or study of SLE PROs[2,3,5]. Test–retest reliability for PROMIS CATs was good (ranging from 0.72 to 0.88), as was their convergent validity when tested against corresponding generic and SLE-specific PRO domains. Correlations between PROMIS CATs and disease activity measures were poor or absent, in line with previous reports[6]. Floor and ceiling effects (scores at the minimum or maximum of the scale, respectively, which can limit the information the scale can provide) were substantially lower for PROMIS CATs compared with the two legacy tools that were used (generic (SF-36) and lupus-specific (LupusQoL))[1]. Kasturi *et al.* concluded that PROMIS CATs are well-suited for use at the point of care in SLE because of their favourable performance characteristics and decreased burden on survey responders[1].

This study[1] illustrates the advantages and disadvantages of using PROMIS CATs with patients with SLE. The lack of correlation with physician assessment (not statistically significant) highlights the uniqueness of PRO data and supports the need for PRO assessment in SLE. Despite the premise of having a low burden on respondents, each respondent was presented with an average of 72.8 questions, although several QOL domains pertinent to SLE were still missing[1,2] (including body image, planning, intimate relationships, procreation and medication concerns). Although PROMIS CATs seemed to have fewer floor and ceiling effects in comparison to the legacy tools utilized in this study[1], the same was not observed for PROMIS short-forms in another study[4], although this difference could be a result of patients being in better health at the time of questionnaire completion in the latter study.

In some health care settings, administration of PROMIS CATs to patients with SLE could be logistically difficult, as use of this tool would entail investments in equipment, time during the encounter and personnel resources. To be useful, the scores would also need to be integrated into the care process. In addition, CATs are only available in English and Spanish versions, so some patients might be unable to use PROMIS in this format.

In the study by Kasturi *et al.*[1], although patients were recruited at point of care, only 20% completed their surveys onsite at the time of visit, with the rest completing their surveys remotely using a computer or smart device on the same day or at a later date. Such remote completion might introduce bias owing to socio-economic status (access to a computer or smart device), age, sex, education or health status. These concerns are substantiated by the statistically significant differences observed in the scores of PROMIS short-forms between those completed online or on paper by patients with SLE[3]. Respondents to paper questionnaires were older, less well-educated and reported worse health status than respondents to online questionnaires, and the differences in PROMIS scores remained statistically significant after adjustment for these covariates[3]. In addition, when using PROMIS CATs, the time to completion of surveys taken remotely might be variable and lag behind the original visit, potentially confounding the validity of criteria computed against disease activity assessment scores obtained during the original visit.

> “it might be advisable to combine generic measures … with an SLE-specific [patient-reported outcome] measure”

Further studies of PROMIS CATs are needed to determine its place in the clinical care of patients with SLE. The use of 14 PROMIS CAT domains might not be necessary to adequately assess QOL, but more research is required to identify which domains are the most relevant and useful. The way in which scores are interpreted needs to be examined among patients and physicians to ensure that the presentation of data to both groups is appropriate. Longitudinal studies in patients with SLE who have varied health and socio-demographic characteristics are also needed to further evaluate the measurement properties, feasibility and performance of PROMIS CATs in a clinical care setting, as well as its responsiveness to change.

Finally, to gain the most comprehensive information from patients, it might be advisable to combine generic measures, such as a selection of PROMIS domains, with an SLE-specific PRO measure such as the LupusPRO[1], LupusQoL[2] or Lupus Impact Tracker (LIT; 10 items)[7] (see Competing interests statement below). These disease-specific PROs include domains pertinent to SLE and have undergone extensive cross-cultural validation studies and psychometric evaluation. The LupusPRO (and its derivative, the LIT) was developed in accordance to the FDA PRO guidelines[8]. All three SLE-specific PROs are responsive to changes in self-reported health status; LupusPRO and LIT are responsive to changes in disease activity; and LIT is responsive to the composite SLE responder index (SRI)[9].

All in all, PROMIS seems to hold promise as a generic tool for measuring QOL in SLE when used in combination with a disease-specific PRO tool, but continued research is needed to identify the best-suited domains and practices for its use.

Meenakshi Jolly is at Rush University Medical Center, 1611 West Harrison Street, Suite 510, Chicago, Illinois 60612, USA.

Patricia Katz is at the University of California San Francisco, 3333 California Street, San Francisco, California 94143, USA.

Correspondence to M.J.
Meenakshi_Jolly@rush.edu

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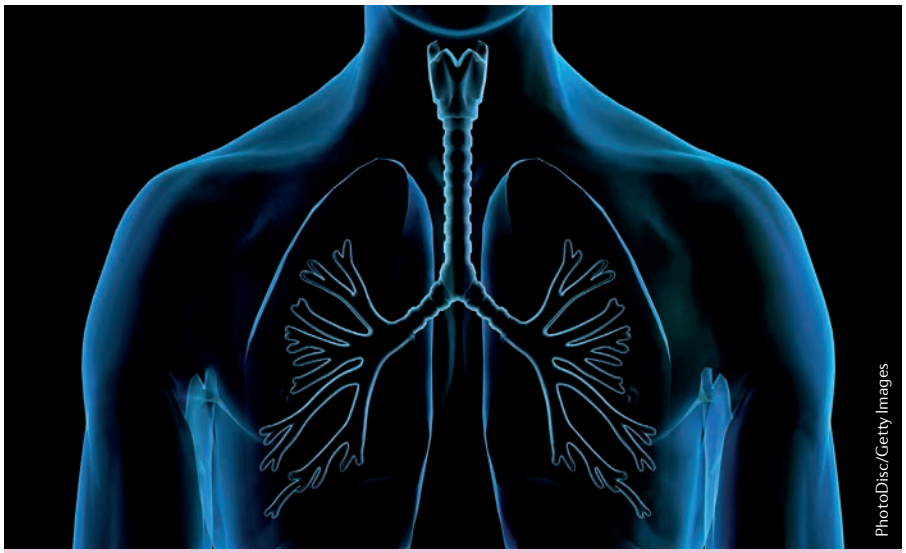
M.J. declares that she is the inventor of LupusPRO and Lupus Impact Tracker, the copyrights for which are owned by Rush University Medical Center and the University of Illinois at Chicago, Illinois, USA. P.K. declares no competing interests.



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SYSTEMIC SCLEROSIS

Choosing patients wisely when treating interstitial lung disease

Richard M. Silver

Systemic sclerosis-associated interstitial lung disease (SSc-ILD) requires accurate diagnosis and staging to identify patients with the highest risk of disease progression, who might benefit from treatment with immunosuppressants. New insights into predictors of mortality in patients with SSc-ILD should improve patient care and inform the design of future clinical trials.

Refers to Goh, N. S. *et al.* Short term pulmonary function trends are predictive of mortality in interstitial lung disease associated with systemic sclerosis. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40130 (2017)

Although trends in survival might have improved[1], the management of disease in patients with systemic sclerosis (SSc; sometimes known as scleroderma) remains a challenge. Unfortunately, in the 35 years since the introduction of captopril (an angiotensin-converting enzyme (ACE) inhibitor) as the first effective treatment for scleroderma renal crisis, we have not witnessed another therapeutic breakthrough of such magnitude. Despite efforts, major advances in the treatment of SSc-associated interstitial lung disease (SSc-ILD) and SSc-associated pulmonary arterial hypertension (SSc-PAH), the two leading causes of SSc-related deaths[2], have not been achieved. Even with the expanding pharmacologic armamentarium for PAH, patients with SSc-PAH generally do not respond as well to treatment as do patients with idiopathic PAH[3]. Moreover, reports of successful treatment of SSc-ILD with immunosuppressive agents[4–6] must be regarded as being of only marginal benefit. Do these results truly reflect the performance of the drugs under investigation, or was the magnitude of improvement shown in these trials diminished by the patients chosen for each study? New research by Goh *et al.*[7] highlights the importance of patient selection when it comes to planning clinical trials in SSc-ILD.

Published a decade ago, the Scleroderma Lung Study (SLS-I) was the first randomized controlled trial (RCT) to demonstrate a statistically significant effect ($P<0.03$) of immunosuppressive therapy (oral cyclophosphamide) on forced vital capacity (FVC) in patients with SSc-ILD, although the improvement in FVC was of minimal clinical significance[4]. The 2006 Fibrosing Alveolitis in Scleroderma Trial (FAST) study demonstrated a similarly small effect using monthly intravenous cyclophosphamide followed by oral azathioprine[5]. In the 2016 SLS-II study, mycophenolate mofetil was shown to be as effective as and better tolerated than cyclophosphamide, yet the adjusted improvement in the percentage predicted FVC from baseline to 24 months was only 2.19 for the mycophenolate mofetil group (95% CI 0.53–3.84) and 2.88 for the cyclophosphamide group (95% CI 1.19–4.58)[6]. Notably, some patients in each of these clinical trials[4–6] demonstrated a greater degree of improvement in FVC than is captured by the averaged results. In addition, greater improvements in FVC were also observed in an earlier, open-label trial of cyclophosphamide in which patients were selected on the basis of declining FVC[8]. It seems likely, therefore, that the efficacy of immunosuppressive therapy for SSc-ILD has been understated as a result of clinical trial design, providing a strong argument for the careful selection of appropriate patients in future trials.

Indeed, a *post hoc* analysis of existing RCT data[9] and an analysis of clinical parameters and survival from a large UK cohort[7] support this notion. Retrospective analysis of SLS-I data identified the severity of reticular infiltrates on baseline high resolution computed tomography (HRCT) chest scans and the baseline modified Rodnan skin score to be predictive of responsiveness to cyclophosphamide therapy[9]. In a subsequent report, researchers postulated that such *post hoc* analysis might have a substantial effect on clinical trial design, and that such data could be used to enrich patient recruitment in future trials, to calculate sample sizes and to judge the feasibility of a trial[10].

Following this report, the 2017 study by Goh and colleagues provides an important step forward in selecting patients for

future SSc-ILD clinical trials[7]. Utilizing a well-characterized cohort of 162 patients with SSc-ILD with a median follow-up of 155 months, the authors examined the relationships between pulmonary function trends at 12 months and at 24 months against 15-year survival[7]. As expected, baseline physiologic measures of lung function such as FVC, diffusing capacity of the lung for carbon monoxide (D_{LCO}), transfer coefficient (K_{CO}) and FVC/D_{LCO} ratio, as well as categorization of patients based on FVC and extent of fibrosis on HRCT imaging, were independent determinants of mortality. Somewhat surprisingly, however, sex, smoking status, Scl-70 autoantibody positivity, type of SSc (limited cutaneous or diffuse cutaneous) and duration of SSc were not predictive of mortality[7].

> “By carefully selecting patients, we can better judge the efficacy of potential therapies …”

Two important findings have emerged from the study by Goh *et al.*[7]. First, at 12 months, the change in FVC provided the most accurate prognostic information when expressed as either a decline of ≥10% in FVC from baseline or a marginal (5–9%) decline in FVC in association with a ≥15% decline in D_{LCO}. Using a severity staging system that integrated disease extent as seen by HRCT with FVC levels, the prognostic value of pulmonary function trend in the whole cohort was entirely ascribable to disease progression in patients with ‘extensive fibrosis’ at this time point[7]. The authors have thus identified the subset of SSc-ILD patients at the highest risk for disease progression, who should be the strongest candidates for inclusion in future clinical trials.

A second important finding is that at 24 months, changes in FVC were not predictive of survival, whereas changes in gas exchange parameters (D_{LCO}, K_{CO} and FVC/D_{LCO}) were of prognostic significance[7]. The authors conclude that the linkage between trends in gas exchange and mortality is likely to reflect the progression of ILD, as well as the progression of pulmonary vasculopathy independent of the ILD[7]. Such information might suggest the need to incorporate pulmonary vasoactive therapies in future trials for SSc-ILD.

Knowledge gained from the study by Goh *et al.*[7] should strongly influence the selection of patients for future SSc-ILD clinical trials. These findings also support the use of serial spirometry and gas exchange testing as important aspects in the clinical monitoring of patients with SSc. By carefully selecting patients, we can better judge the efficacy of potential therapies while also avoiding potentially risky and unnecessary therapies for patients not likely to derive benefit from them.

Richard M. Silver is at the Medical University of South Carolina, 96 Jonathan Lucas Street, MSC 637, Charleston, South Carolina 29425, USA.
silverr@musc.edu

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SYSTEMIC LUPUS ERYTHEMATOSUS

BAFF emerges from the genetic shadows

William Stohl

A newly identified insertion–deletion variant of the B cell activating factor (BAFF)-encoding *TNFSF13B* gene leads to increased levels of soluble BAFF and is associated with the development of systemic lupus erythematosus. The discovery raises a number of compelling questions for further investigation.

Refers to Steri, M. *et al.* Overexpression of the cytokine BAFF and autoimmunity risk. *N. Engl. J. Med.* 376, 1615–1626 (2017)

The *TNFSF13B* gene codes for B cell activating factor (BAFF, also known as B lymphocyte stimulator (BLyS)), a 285-amino acid type II transmembrane protein member of the TNF ligand superfamily (member 13B)[1,2]. Circulating BAFF levels are commonly elevated in patients with systemic lupus erythematosus (SLE) and correlate with clinical disease activity[3–5]. Therapeutic neutralization of BAFF with the anti-BAFF monoclonal antibody belimumab is efficacious in many patients with SLE[6,7], and belimumab is the only drug to have been approved by the FDA for use in SLE in the past 60 years[8]. Given that BAFF is the molecular target of the only biologic agent approved for the treatment of SLE, one could reasonably expect *TNFSF13B* to be an SLE susceptibility gene. Nevertheless, despite a myriad of genome-wide association studies (GWAS) that collectively have identified a growing list of genetic loci (currently >50) associated with SLE[9], *TNFSF13B* had, remarkably, been conspicuously absent from this list — until now. With the recent publication by Steri *et al.*[10], *TNFSF13B* takes its rightfully anticipated spot on the list.

How did Steri *et al.* succeed in identifying *TNFSF13B* as an SLE susceptibility gene when others had failed? As is often the case in success, the recipe consisted of a good plan, good luck and good insight. The good plan was driven by the goal of identifying disease-related endophenotypes for an autoimmune disease in an unbiased manner. Of note, multiple sclerosis, rather than SLE, was the initial target disease. Rather than studying broad-based populations as commonly done in GWAS, the investigators initially focused their attention on a restricted population (Sardinians), which, in all likelihood, is considerably less heterogeneous than the cosmopolitan populations routinely analysed through GWAS. The investigators leveraged their large case–control sets of patients with multiple sclerosis and healthy volunteers (all from Sardinia) and applied them to a highly dense genetic map constructed from 2,120 Sardinians who had previously undergone whole-genome sequencing and to a highly refined map at the *TNFSF13B* locus that includes insertion–deletion variants. With these tools, Steri *et al.*[10] identified a GCTGT→A variant, which they named 'BAFF-var'.

The good luck came from the high frequency of BAFF-var in the Sardinian population (0.288). Given this high frequency, Steri *et al.* were able to demonstrate a strong association between BAFF-var and multiple sclerosis (OR 1.27; $P = 1.23 \times 10^{-9}$). Analysis of a case–control set of individuals from mainland Italy yielded a similar odds ratio (OR 1.25) but, owing to the much lower frequency of BAFF-var in the mainland Italian population (0.063), this analysis lacked robust statistical power. Had the mainland Italian population been analysed without prior analysis of the Sardinian population, the association might have been dismissed as a false positive.

The good insight came in the realization that the association between BAFF and multiple sclerosis should extend to other autoimmune diseases in which B cells have a contributory role. Given the compelling biological evidence in support of a contribution of BAFF to SLE, the investigators turned their attention to SLE. Using case–controls sets from Sardinia, mainland Italy and the Iberian Peninsula, Steri *et al.* documented an association between BAFF-var and SLE (OR 1.44; $P = 6.74 \times 10^{-10}$ in a combined analysis across all samples) that was even greater than that observed between BAFF-var and multiple sclerosis.

The investigators then performed additional studies to connect the genetics of BAFF-var with the biology of BAFF. BAFF-var creates an alternative polyadenylation signal that generates a shorter 3′-untranslated region transcript that lacks a binding site for inhibitory microRNAs. Reduced binding of inhibitory microRNAs to BAFF mRNA would promote increased translation into BAFF protein. Indeed, serum BAFF levels in healthy Sardinian volunteers were found to be significantly greater in those individuals harbouring the BAFF-var genotype than in those individuals harbouring the wild-type genotype.

> “Does the association between BAFF-var and SLE hold across other racial or ethnic populations?”

Moreover, elevated levels of circulating BAFF were observed in healthy donors in whom multiple sclerosis developed up to 12 years later, suggesting that individuals bearing the BAFF-var genotype might be constitutive over-producers of BAFF. Although comparable studies were not performed in healthy donors who developed SLE years later, it makes sense that longstanding increases in circulating BAFF levels could promote development of SLE in hosts with such diathesis.

As is routinely the case for novel and exciting findings, a bevy of additional questions emerges. For one, does the association between BAFF-var and SLE hold across other racial or ethnic populations? The wide disparity between Sardinians and mainland Italians in frequency of BAFF-var (0.288 versus 0.063) suggests that wide disparities could also prevail across discrete racially or ethnically defined populations. It should be enlightening to study the frequency of BAFF-var in populations with a high penetrance of SLE, such as African Americans.

Moreover, are there other BAFF variants that associate with SLE? Now that the *TNFSF13B* gene is a recognized SLE susceptibility gene, there is no *a priori* reason that other variants could not be contributing to SLE susceptibility. Indeed, replicating the study of Steri *et al.* in, for example, the African-American population might reveal one or more such variants.

Whether BAFF-var promotes a specific clinical phenotype or panel of clinical phenotypes also remains to be determined. SLE is not a monolithic clinical disorder; its manifestations are highly protean. A genetic biomarker that permits the physician caring for the SLE patient to accurately anticipate development of certain manifestations (for example, nephritis) and intervene pre-emptively would be invaluable.

Future studies might also investigate whether expression of BAFF-var alters responsiveness to therapeutic agents that target BAFF. In the seminal phase III trials of belimumab, the clinical response rate among patients with SLE treated with belimumab plus standard care was greater than that among patients with SLE treated with placebo plus standard care. Nevertheless, the percentage of patients who did not respond to belimumab therapy in these trials remained disappointingly high (>40%)[6,7]. One cannot help but wonder whether constitutive overproduction of BAFF might have subverted the therapeutic capacity of belimumab in at least some of the non-responders.

> “…are there other BAFF variants that associate with SLE?”

Finally, the results of the study by Steri *et al.* raise the question of whether BAFF-var associates with other systemic autoimmune rheumatic diseases. Increased BAFF expression is not unique to SLE but is a feature of many rheumatic disorders, including Sjögren syndrome, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, giant cell arteritis and others. As with SLE, expression of BAFF-var (or other BAFF variants) might influence clinical presentation and responsiveness to individual treatment modalities.

Overall, the seminal study of Steri *et al.* should serve as a springboard for many lines of fertile investigation.

William Stohl is at the Division of Rheumatology, Department of Medicine, University of Southern California Keck School of Medicine, 2011 Zonal Ave HMR 711, Los Angeles, California 90033, USA.
stohl@usc.edu

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RHEUMATOID ARTHRITIS

The benefits of early treatment after decades

Angela Zink and Katinka Albrecht

Early, targeted treatment improves the outcome of rheumatoid arthritis, reducing disease-associated disability and mortality. Until now, it was unknown whether these beneficial effects extended beyond 10 years following initial therapy. Could these effects persist even after 20 years?

Refers to Gwinnutt, J. M. *et al.* The 20 year outcome and association between early treatment and mortality and disability in an inception cohort of patients with rheumatoid arthritis: results from the Norfolk Arthritis register. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40090 (2017)

An overwhelming amount of evidence supports the idea that in patients with rheumatoid arthritis (RA), early intervention and treatment to a defined target, such as remission or low disease activity, is the best premise for achieving beneficial outcomes[1,2]. Many immunosuppressive drugs are efficacious and achieve high rates of remission when started early in disease[3,4]. In patients with RA, the disease course is thought to be adjustable or even reversible if treatment is started within a limited time window following the onset of symptoms[3]. Starting treatment within this 3–6 month window of opportunity is crucial for achieving the best short and medium term results; early prevention of joint damage is expected to translate into reduced disability in later years. In a new study, Gwinnutt *et al.*[5] highlight the benefits of early treatment in patients with RA over the course of 20 years.

The Norfolk Arthritis register (NOAR) is the world's largest and most comprehensive primary-care-based early arthritis cohort. This registry has substantially contributed to our understanding of the risk factors for arthritis onset and chronification. The present study analysed data from patients enrolled between 1990 and 1994 who were recruited within two years following symptom onset and fulfilled the 2010 ACR–EULAR criteria for RA[5]. Of the 1,098 patients enrolled during this period, 602 met the inclusion criteria for this study.

The main question of this study[5] was whether early treatment with conventional synthetic DMARDs (csDMARDs) would translate into better outcomes regarding disability and mortality over the course of 20 years. A number of studies have shown the beneficial outcomes of early intervention over the course of ≤10 years[4], but to date, no other study has addressed a longer time period.

The authors compared patients who received early csDMARDs (within 6 months of symptom onset; 27% of the cohort) with those who received late csDMARD treatment (≥6 months after symptom onset; 29%), as well as with those who never received any csDMARD treatment (44%)[5]. The baseline clinical features, such as the number of tender and swollen joints or the level of acute phase reactants, were similar in all three groups except for the baseline disability, as measured by Health Assessment Questionnaire (HAQ), which was better in the untreated group. The main difference between those patients treated with csDMARDs and those untreated was the percentage of patients positive for anti-citrullinated protein antibodies (ACPAs) and/or rheumatoid factor; 53–55% and 15% patients were ACPA-positive in the treated and untreated groups, respectively, indicating a higher probability of disease progression in the treated group.

When adjusted for age and sex, the mortality rates were similar between the groups and slightly higher than in the general population, with standardized mortality rates ranging between 1.23 and 1.28. The survival curves of the three groups disconnected after the 10 year time point and seemed to be best in the untreated group, followed by the early treatment group. However, when baseline differences between the groups were fully taken into account, there was a trend towards lower mortality in both the early treatment (OR 0.78, 95% CI 0.54–1.11) and late treatment groups (OR 0.66, 95% CI 0.47–0.92) when compared with the untreated group[5].

Concerning disability, the results were even more convincing. Patients who were treated within 6 months of symptom onset had similar levels of disability to those untreated, and significantly better HAQ scores than those with late onset of treatment, after controlling for confounding by indication. We suspect that the patients in the untreated group had

some form of self-limiting, non-destructive arthritis, as they did not receive any steroids or csDMARDs over the course of 20 years. These patients can henceforth be considered a comparator group, similar to the normal, healthy population. When considering treatment effects, the focus of the comparison should therefore be between the groups with early and late treatment onset. These results imply that long-term disability in patients with RA can be reduced by early treatment, even with poorly efficacious immunosuppressive drugs[5].

When following a cohort of patients over such a long time, the weight of the results strongly depends on the completeness of follow-up. In this study[5], mortality rates could be fully ascertained as all patients were flagged with the Office for National Statistics, who provided copies of their death certificates. Over the course of the study[5], 205 patients (34%) died during the follow-up period and 135 (22%) declined further follow-up visits, whereas the drop-out without any information was rare (8%). Hence, data on 207 patients (34% of the original cohort) were available to be assessed at 20 years, providing sufficient explanatory power for follow-up data.

Given the differences observed between the treatment groups, one might suspect that the treatment during follow-up also differed, with lower proportions of patients receiving biologic agents in the late treatment group. However, the percentages were equally low in both treatment groups (11.9% and 10.8% for the early and late treatment groups, respectively), implying less efficacious treatment strategies were used in both groups than those in use today.

An obvious (and inevitable) limitation of this study[5] is that the drug treatment utilized does not reflect current practice or ideas about adequate early treatment of RA. Of those patients who received treatment within 6 months of symptom onset (the early treatment group), 59% were prescribed sulfasalazine, 28% steroids and only 5% methotrexate. Today, methotrexate is the anchor drug among csDMARDs, and is used in the vast majority of cases as a first-line DMARD[4]. However, methotrexate was only introduced into rheumatologic treatment in the early 1980s, when sulfasalazine had been available for decades[6]. Therefore, the fact that patients who were mainly treated by general practitioners preferentially received sulfasalazine treatment is not astonishing. These results demonstrate the important finding that even when patients with RA are treated with a drug of limited effectiveness, the benefits of early treatment can still be seen after 20 years.

In the past two years, two observational studies have independently demonstrated that despite the increase in remission rates owing to increased treatment intensity over the past decade, disability has not been comparably reduced[7,8]. These findings put the results of the NOAR study[5] into perspective, supporting the notion that early treatment in RA can reduce disability. Considering the increasing availability of efficacious therapies, disability resulting from RA could eventually belong to the past.

Cardiovascular and overall mortality rates in patients with RA have declined in the past few decades[9,10]. The results from the NOAR cohort[5] indicate that besides treatment intensity, early intervention might be one factor accountable for improved mortality in RA, and show that early intervention pays back dividends even after two decades. These findings should encourage general practitioners and rheumatologists to strive for even earlier detection and treatment of patients with this still potentially disabling disease.

Angela Zink and Katinka Albrecht are at the German Rheumatism Research Centre (DRFZ), Epidemiology Unit, Charitéplatz 1, 10117 Berlin, Germany.

Correspondence to A.Z.
zink@drfz.de

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PAEDIATRIC RHEUMATIC DISEASE

What is the best definition of clinical remission in JIA?

Gabriella Giancane and Angelo Ravelli

The optimal definition of clinical remission in juvenile idiopathic arthritis is still uncertain. A new study has found that the current criteria for clinically inactive disease do not always identify the same group of patients. Why is there such a discrepancy? And which approach to defining remission is the most advantageous?

Refers to Shoop-Whorral, S. J. W. *et al.* How common is clinically inactive disease in a prospective cohort of patients with juvenile idiopathic arthritis? The importance of definition. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210511 (2017)

In the past two decades, the management of juvenile idiopathic arthritis (JIA) has been revolutionized by the shift towards early aggressive interventions, the development of new combination treatment strategies and the introduction of biologic response modifiers[1]. These advances have considerably increased the likelihood of achieving disease remission or, at least, minimal levels of disease activity in patients with JIA and have consequently moved therapeutic aims towards the attainment of complete disease quiescence[2]. Implementation of a treat-to-target strategy aiming for disease remission has been suggested for routine paediatric rheumatology practice[3]; however, the optimal definition of clinical remission in JIA is still unclear, as highlighted by a new study from Shoop-Whorral and colleagues[4]. In this study, the authors compared the frequency of minimal disease activity (MDA) and clinically inactive disease (CID) across different criteria in a cohort of children with JIA

Clinical tools are needed that enable precise measurement of disease remission in JIA. Two categories of measures are currently available for this purpose: definitions based on multiple criteria, and composite disease activity scores. The former group includes the Wallace criteria for CID[5,6] and the Magni-Manzoni criteria for MDA[7], whereas the latter is centred on the Juvenile Arthritis Disease Activity Score (JADAS)[8]. The JADAS is a composite disease activity index based on four individual measures: physician's global assessment of disease activity, parent's and/or patient's assessment of child's well-being, count of joints with active arthritis (assessed in 71, 27 or 10 joints, depending on the version) and erythrocyte sedimentation rate (ESR). Different versions of JADAS have been validated, including a three-item version (the so-called clinical JADAS, cJADAS)[8]. However, which of these two types of approaches — criteria or composite disease activity score — is more advantageous is still unclear.

To address this question, Shoop-Whorral and co-workers[4] compared the performance of published criteria for defining CID and MDA in a large inception cohort of 1,415 children with JIA assessed at 1 year following presentation. The authors found that the majority of patients had evidence of persistently active disease at the study endpoint, with an overall CID frequency of ~30% and MDA frequency of ~50%; however, the measured frequency of both states varied greatly between criteria. The Wallace definition proved to be the most stringent criteria, with 25% of children classified as having CID; whereas 38% of children were categorized as having CID by the JADAS. The main matter of concern was the poor overlap (only 44% of patients) between the Wallace criteria and the JADAS when assessing CID (FIG. 1). This finding led the authors to conclude that currently used targets intended to capture the same disease state identify diverse groups of children. Thus, the use of different criteria to define CID or MDA in clinical practice could potentially lead to overtreatment or undertreatment.

Looking at the study data, the most likely explanation for the poor concordance between the Wallace and JADAS criteria was the role of the parental global assessment of the child's well-being, which is included in the JADAS and not in the Wallace criteria. Indeed, the median parental global assessment scores in the patients meeting only the Wallace criteria (2.2 cm of the 10 cm visual analogue scale (VAS)) and in those meeting only the JADAS criteria (0.0 cm) were markedly different. Because pain is a major determinant of the parental global assessment and children with chronic arthritis might have persistent pain symptoms independent of joint inflammation, the authors argued that the parental global assessment might incongruously inflate the JADAS score, making it an imprecise measure of remission[4]. However, many studies have shown that physicians and parents often differ in their estimation of JIA activity, and it cannot be assumed that the physician's assessment is the correct one. In addition, a parents' perception of disease burden can vary across ethnic and cultural environments[8]. Further studies are needed to investigate whether the use of the parental global assessment is a limitation in the JADAS. Nevertheless, we believe it is important to integrate the parents' and, whenever possible, children's perspective into clinical assessment as it helps with physician's choices and improves adherence to treatment.

Another problem that might have affected concordance between criteria was the tendency for some physicians not to mark the VAS at exactly zero even on absence of disease activity. This drawback was probably due, at least in part, to the relative aversion to extremes that is often seen when using the horizontal line VAS, with very low values (0.1 or 0.2 cm) being frequently obtained when the assessor actually intended to mark the end of the line. The use of the 21-numbered circle VAS in 0.5-unit increments might obviate this limitation and increase the measurement precision[9].

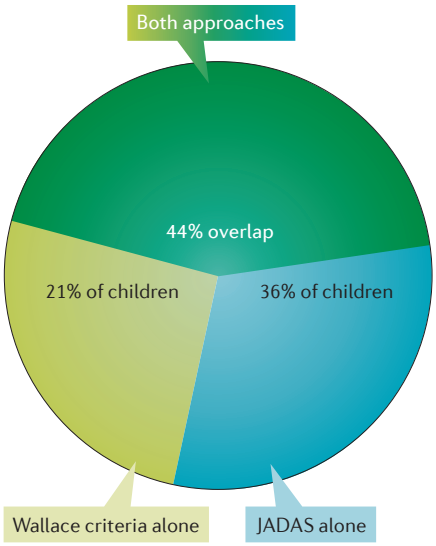

Figure 1 | **Categorizing clinically inactive disease in children with juvenile idiopathic arthritis.** For identifying those children with juvenile idiopathic arthritis who have clinically inactive disease (CID), the Wallace criteria and JADAS seem to capture different groups of patients. Of those children assessed in Shoop-Whorral and colleagues' study who were categorized as having CID by either the Wallace criteria or JADAS[4], only 44% met both approaches. Information for this figure obtained from Shoop-Whorral, S.J.W. *et al.* How common is clinically inactive disease in a prospective cohort of patients with juvenile idiopathic arthritis? The importance of definition. *Ann Rheum Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210511 (2017)

A further explanation could be the stringency of the Wallace criteria, which require the physician global assessment to be at the lowest level of the scale used (that is, 0 of the 0–10 VAS scale). The fact that in some therapeutic studies these criteria have been modified and the minimum score of the physician global assessment set to 1 or even 2 (REF. 2) is indirect evidence that the original definition is infrequently met, at least in the short time frame of a clinical trial. Lastly, increased acute phase reactants due to a non-rheumatologic cause despite the absence of active joints might have precluded fulfilment of both the Wallace and the JADAS criteria, but not the three-variable version of the JADAS definition (cJADAS), as this tool does not incorporate an inflammatory marker[10].

When interpreting the findings of Shoop-Worral *et al.*, some caveats should be borne in mind[4]. The amount of missing data was considerable. To give an example, the physician and parental global assessments were available for only around two thirds of the patients, and measurements of acute phase reactants for >20% of the patients. The absence of this information was accounted for using several assumptions and multiple imputations; however, estimates after imputation were substantially higher than those from complete case analysis, in which the Wallace and JADAS remission rates dropped to 4.5% and 5.1%, respectively. These figures seem quite low for a routine patient population treated with contemporary care. The preliminary Wallace criteria were used instead of their revised version, the latter of which includes the parent report of morning stiffness. The face validity of the various definitions was not compared with reference to external criteria, such as a physician's or parent's subjective assessment of remission or the treatment decisions made at the visit. Finally, the study design did not allow for analysis of the relationship between clinical criteria and remission defined by imaging or biomarkers.

Despite these limitations, the study of Shoop-Worral and co-workers[4] is important as it calls for future studies aimed to further scrutinize the validity of criteria for JIA activity states in different clinical and research settings and in patients of diverse ethnicity. These analyses should include a comparison of the ability of targets to predict long-term outcomes. The achievement of these goals will enable the physician to better compare patient populations and to analyse the effectiveness of current and novel therapeutic protocols.

Gabriella Giancane and Angelo Ravelli are at the Università degli Studi di Genova and Istituto Giannina Gaslini, Via G. Gaslini 5, 16147 Genoa, Italy.

Correspondence to A.R.
angeloravelli@gaslini.org

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The authors declare no competing interests.

Synovial tissue research: a state-of-the-art review

Carl Orr[1], Elsa Sousa[2], David L. Boyle[3], Maya H. Buch[4], Christopher D. Buckley[5], Juan D. Cañete[6], Anca I. Catrina[7], Ernest H. S. Choy[8], Paul Emery[4], Ursula Fearon[9], Andrew Filer[5], Danielle Gerlag[10,11], Frances Humby[12], John D. Isaacs[13], Søren A. Just[14], Bernard R. Lauwerys[15], Benoit Le Goff[16], Antonio Manzo[17], Trudy McGarry[9], Iain B. McInnes[18], Aurélie Najm[16], Constantino Pitzalis[12], Arthur Pratt[13], Malcolm Smith[19], Paul P. Tak[10,20], Rogier Thurlings[21], João E. Fonseca[2] and Douglas J. Veale[1]

Abstract | The synovium is the major target tissue of inflammatory arthritides such as rheumatoid arthritis. The study of synovial tissue has advanced considerably throughout the past few decades from arthroplasty and blind needle biopsy to the use of arthroscopic and ultrasonographic technologies that enable easier visualization and improve the reliability of synovial biopsies. Rapid progress has been made in using synovial tissue to study disease pathogenesis, to stratify patients, to discover biomarkers and novel targets, and to validate therapies, and this progress has been facilitated by increasingly diverse and sophisticated analytical and technological approaches. In this Review, we describe these approaches, and summarize how their use in synovial tissue research has improved our understanding of rheumatoid arthritis and identified candidate biomarkers that could be used in disease diagnosis and stratification, as well as in predicting disease course and treatment response.

Fibroblast-like synoviocytes (FLSs). Also known as type B synovial lining cells, FLSs account for the majority of cells in the synovial lining layer.

[1]*Centre for Arthritis and Rheumatic Disease, University College Dublin, Dublin Academic Medical Centre, St. Vincent's University Hospital, Elm Park, Dublin 4, Ireland.*

Correspondence to D.J.V.
douglas.veale@ucd.ie

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Chronic inflammatory arthritides comprise a heterogeneous group of diseases that are characterized by inflammation of the synovium, which is often accompanied by the destruction of adjacent cartilage and bone. Inflammation is characterized by synovial neovascularization, stromal proliferation and leukocyte extravasation[1]. For the purpose of this Review, we focus on rheumatoid arthritis (RA), owing to its prevalence and the fact that this disease is the most extensively studied and most common cause of synovitis. RA is usually persistent and progressive, and leads to joint damage, disability and deformity if left untreated. The disease is associated with a reduction in quality of life, as well as with decreased longevity, and represents an important burden on health care spending[2–4].

Within the past two decades, several considerable advances have been made in the treatment of inflammatory arthritides in general, and particularly in the treatment of RA. However, further progress is needed. The patterns of clinical response to treatment are remarkably similar for agents with different targets, and this finding challenges our current understanding of disease mechanisms. In addition, despite the aforementioned unprecedented progress, a substantial proportion of patients with RA still do not achieve a state of low disease activity or remission following treatment[5,6].

The main challenges in biomedicine and translational research in RA are early diagnosis, personalized medicine and the development of meaningful outcome assessments[7]. A logical hypothesis is that each of these aims can be facilitated by the identification and development of appropriate biomarkers. However, although peripheral blood biomarkers such as rheumatoid factor and anti-citrullinated protein antibodies (ACPAs) have been shown to be relatively specific and might predict the development of RA in asymptomatic individuals[8], they are reportedly found in only 70–80% of patients with RA[9]. Indeed, beyond rheumatoid factor and ACPAs, our repertoire of blood biomarkers to assist with diagnosis and to provide insights into disease progression and response to therapy is currently extremely limited[10,11].

As the synovium is the principal target of inflammation in RA, and the resident fibroblast-like synoviocytes (FLSs) are implicated in the pathogenesis of synovitis, one promising approach could be to search for biomarkers in inflamed synovial tissue. Using a combination of established methodologies, together with new high-throughput omics technologies that have the capability to examine the expression of genes and their products on a scale never before possible (BOX 1), a new opportunity awaits in the search for these biomarkers. This Review summarizes

Key points

- Synovial tissue is the target tissue for autoimmune arthritides such as rheumatoid arthritis.
- Synovial biopsy is a safe and well-tolerated procedure that is becoming more widely available.
- There is a significant body of work from the past 30 years analysing the cellular and molecular changes in synovial tissue from patients with rheumatoid arthritis to identify specific biomarkers.
- Technological advances in molecular and cellular analysis now provide new opportunities for defining new biomarkers and targets.

Author addresses

[1]Centre for Arthritis and Rheumatic Disease, University College Dublin, Dublin Academic Medical Centre, St. Vincent's University Hospital, Elm Park, Dublin 4, Ireland.
[2]Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, 1649–035, Lisbon, Portugal.
[3]University of California San Diego School of Medicine, 9500 Gilman Drive, La Jolla, California 92093, USA.
[4]Leeds Musculoskeletal Biomedical Research Unit, University of Leeds, Chapel Allerton Hospital, Chapeltown Road, Leeds LS7 4SA, UK.
[5]Rheumatology Research Group, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.
[6]Arthritis Unit, Rheumatology Department, Hospital Clínic, IDIBAPS, Villarroel 170, 08036 Barcelona, Spain.
[7]Rheumatology Unit, Department of Medicine (Solna), Karolinska Institute and Karolinska University Hospital, 171 76 Stockholm, Sweden.
[8]Cardiff University School of Medicine, Institute of Infection and Immunity, 1 st Floor, Tenovus Building, Heath Park, Cardiff CF14 4XN, UK.
[9]Department of Molecular Rheumatology, Trinity College Dublin, University of Dublin, College Green, Dublin 2, Ireland.
[10]Department of Clinical Immunology & Rheumatology, Amsterdam Rheumatology and Immunology Centre, Academic Medical Centre, University of Amsterdam, Room F4-105, PO Box 22700, 1100 DE, Amsterdam, Netherlands.
[11]Clinical Unit Cambridge, GlaxoSmithKline, Cambridge, UK.
[12]Centre for Experimental Medicine and Rheumatology, John Vane Science Centre, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK.
[13]Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, UK.
[14]Department of Medicine, Svendborg Hospital, Odense University Hospital, Valdemarsgade 53, 5700 Svendborg, Denmark.
[15]Université catholique de Louvain and Department of Rheumatology, Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, 1200 Bruxelles, Belgium.
[16]Rheumatology Unit, Nantes University Hospital, Hôtel-Dieu, 1 place Alexis-Ricordeau, 44093 Nantes cedex 1, France.
[17]Rheumatology and Translational Immunology Research Laboratories (LaRIT), Division of Rheumatology, IRCCS Policlinico San Matteo Foundation/University of Pavia, P.le Golgi 19, 27100 Pavia, Italy.
[18]Institute of Infection, Immunity and Inflammation, College of Medicine, Veterinary and Life Sciences, University of Glasgow, 120 University Avenue, Glasgow G12 8TA, UK.
[19]Rheumatology, Flinders University, GPO Box 2100, Adelaide 5001, South Australia, Australia.
[20]GlaxoSmithKline, Cambridge, UK.
[21]Institute for Molecular Life Sciences, RadboudUMC, Theodoor Craanenlaan 11, Nijmegen 6525 GA, Netherlands.

how synovial tissue research has advanced our understanding of RA, contributed to progress in addressing key challenges in the field and identified candidate biomarkers (TABLE 1). We first briefly discuss the anatomy and physiology of the healthy synovial joint, the main changes that occur in the inflamed joint, and current approaches to biopsy retrieval and analysis.

Synovial joint anatomy and physiology

The synovial joint comprises opposing bones with articular surfaces that are covered by cartilage. The main protein in bone is type I collagen, whereas cartilage comprises mainly type II collagen and proteoglycan molecules. The non-articulating surfaces of synovial joints are lined by a thin adventitious layer known as the synovium. Normal synovial tissue comprises one to three layers of specialized columnar FLSs that are interspersed with macrophages[12]. The entire structure is enclosed by a fibrous capsule and, together with ligaments, muscles and tendons, the fibrous capsule confers strength and stability to the joint.

Several factors contribute to the maintenance of normal homeostasis in the synovial joint, including the expression of the protective lubricin[13], the secretion of matrix metalloproteinases (MMPs) by FLSs, the immune sentinel roles of resident macrophages and FLSs, the regulated entry and exit of leukocytes involved in immune surveillance, and local regulation by cytokines and growth factors.

Cytokines and growth factors are important regulators of FLSs and chondrocytes[14–16]. Cytokines are categorized as either pro-inflammatory or anti-inflammatory depending on their immediate effects on specific tissues, although considerable potential exists for pleiotropism depending on the cells targeted and the microenvironment. Cytokines and growth factors are ubiquitous in the synovium and synovial space, and originate either from the plasma or from FLSs, chondrocytes and cells in the surrounding tissues[16].

The joint is a dynamic environment that is subject to minor trauma continually — owing to movement and, in some joints, compression due to weight bearing — and is therefore subject to continued wound healing and repair processes. Continual remodelling of the articular cartilage and adjacent bone is therefore necessary, and this process requires a balance of anabolic and catabolic enzyme activity in both cartilage and bone. Carefully regulated proteolytic enzymes are responsible for maintaining the balance between anabolic and catabolic processes within the joint and cartilage[17]. The collagenases MMP1 (interstitial collagenase), MMP3 (stromelysin-1), MMP8 (neutrophil collagenase), MMP13 (collagenase 3) and MMP18 (also known as MMP19) are the most important of these enzymes, as they are the only known enzymes that can directly cleave collagen at a neutral pH[18], but other MMPs contribute to collagen degradation once its triple helix structure has become unravelled[19].

Serine and cysteine proteinases are required to activate pro-MMPs (that is, MMP precursors) after they are secreted. Furthermore, inhibitors of these proteinases

Box 1 | **Synovial tissue research and the omics approach**

The widespread availability of synovial tissue biopsy procedures and analytical methods throughout the world[52] will inevitably enable a targeted approach to the identification of synovial biomarkers. The advent of new proteomic, transcriptomic and genomic technologies, and the ability to combine clinical and radiological markers with these technologies, will facilitate progress in this area. The omics approach has been usefully applied to the identification of key players and protein interactions in several diseases. Studying the genome, RNA expression or protein expression each have different biases, and combined approaches could arguably lead to a more accurate assessment of important protagonists[104].

Proteomics offers the advantage that the functional units (that is, the proteins) of the cell are being studied directly, and this approach is likely to provide information that most accurately reflects what is actually happening in the synovium. Technologies such as SomaLogics that have the power to measure thousands of proteins in small volumes of tissue have the potential to enable a more complete characterization of the protein networks that underlie diseases such as rheumatoid arthritis (RA)[160]. Furthermore, new technologies for protein separation, processing and identification are expected to increase proteome coverage. In RA, the proteomics approach has so far focused on peripheral blood mononuclear cells, serum and synovial fluid[66,67,139]; the possibility that the synovial tissue itself might hold the key to elucidating the underlying disease-associated protein networks has yet to be fully exploited by proteomic studies.

In relation to transcriptomic analysis, microarray technology has, to date, been the most frequently used strategy in the field of biomarker research. This technology facilitates the identification of candidate genes that are involved in pathophysiological processes. However, gene expression levels do not always predict protein levels owing to transcriptional and translational regulatory mechanisms and the activity of protein-degradation processes[17].

Microarrays contain probes for thousands of different genes, and they are suitable for screening large cohorts; however, the high-throughput techniques used in transcriptomics also enable the detection of significant gene-expression differences within modestly sized cohorts[161]. Transcriptomic analysis is already being used to examine the gene signatures of synovial tissue, and such investigation is augmented by the newer sequencing technologies that enable deeper transcriptional coverage than do microarrays and that include spliced variants. Several studies have demonstrated that microarray technology is a useful and practical methodology for studying gene expression in RA, and have characterized gene-expression patterns in the synovia of patients with RA (see the section of the main text entitled 'Gene-expression profiles')[162,163].

(such as tissue inhibitors of metalloproteinases (TIMPs) and inhibitors of serine proteinases (SERPINs)) are also present in the normal joint[20]. The levels and activity of these enzymes can be monitored indirectly by measuring their degradation products in the synovial fluid[21].

Features of the inflamed joint

The inflamed synovium has been studied at the macroscopic, microscopic and molecular levels. The synovium is the primary target of disturbed immunomodulatory pathways in RA. Rheumatoid synovial tissue appears to be macroscopically hyperplastic and hypervascular (FIG. 1a,b), while microscopic analysis reveals hyperplasia of the intimal lining layer (FIG. 1c) and the accumulation of inflammatory cells (FIG. 1d), including T and B lymphocytes, plasma cells, macrophages, neutrophils, mast cells, natural killer cells and dendritic cells, in the synovial sublining[22]. Like the target organs in other autoimmune diseases (for example, Sjögren syndrome and autoimmune thyroiditis), infiltrating T cells and B cells have been demonstrated to form aggregates that have varying degrees of organization and the potential to produce disease-specific ACPAs[23,24].

Intimal lining layer
The lining of the synovium comprising a few cells without a basement membrane and which covers the nonarticular surface of the joint capsule.

Synovial sublining
A loose connective tissue that lies beneath the intimal lining of the synovium.

Pannus
A 'tumour-like' mass of hyperplastic synovial tissue that expands into the joint, invading into bone and cartilage.

Angiogenesis accompanies this immune cell accumulation, but it occurs in an abnormal manner that results in different patterns of blood vessels in different inflammatory arthropathies[25]. The new blood vessels seem to be in an immature state[26]. They permit increased leukocyte migration, and the synovial tissue transforms into an invading pannus that can cause cartilage and bone destruction[27,28]. Despite the increased vascular supply, marked hypoxia has been demonstrated in inflamed synovial membranes *in vivo*[29]. The low tissue partial oxygen pressures in inflamed synovial joint tissue are associated with significant increases in macroscopic synovitis scores and markers of microscopic inflammation, such as $CD68^+$ macrophages and $CD3^+$ T cells in the sublining, and various pro-inflammatory mediators (including TNF, IL-1β, IFNγ and the chemokine macrophage inflammatory protein 3α (MIP3α; also known as CCL20)). When primary synovial fluid cells are exposed *in vitro* to partial oxygen pressures similar to those in inflamed joints, cell migration is significantly increased, suggesting that hypoxia drives pathological changes in the synovium[26].

Many of the pathological changes in inflamed synovial tissue are reflected in the synovial fluid, which has also been studied intensively. Inflammation alters the permeability of synovial tissue[30]; in the RA-affected synovium, the permeability to large molecules is increased, but that to small molecules (for example, urea and glucose) is decreased, an effect that is attributable to a combination of increased vessel permeability, cellular infiltration and synovial hyperplasia. As a result, the total protein content of synovial fluid is higher during inflammation than in the steady state. The molecular weight distribution of the lubrication macromolecule hyaluronic acid is also altered during inflammation, with a shift towards lower molecular weight forms in RA[31]. In addition, rheumatoid joints show increased loss of hyaluronic acid compared with healthy joints, and the mean hyaluronic acid concentration is lower in synovial fluid samples from patients with osteoarthritis (OA) or RA than in those from healthy controls[32,33].

Synovial fluid samples from patients with inflammatory arthritides have markedly raised cytokine concentrations[34]. The role of cytokines in initiating and perpetuating the synovial inflammatory response continues to be studied intensively, and has already led to the development of several useful therapeutic agents and to the identification of further potential targets[16]. Changes in the cellular infiltrate of RA-affected synovial tissue have long been recognized to be associated with the clinical course of disease, and have been used to identify specific responses to conventional and biologic DMARDs[35–37].

In summary, the synovial tissue of patients with inflammatory arthritides displays numerous alterations relative to healthy synovial tissue. Thus, the study of synovial tissue is crucial to improving our understanding of these diseases.

Synovial biopsy

The utility of synovial biopsy is clear; the analysis of biopsy-obtained synovial tissue samples has increased our understanding of the pathogenesis of RA, identified potential therapeutic targets, and enabled the evaluation

Table 1 | **Areas in which synovial tissue research has provided insights into rheumatoid arthritis**

Research areas	Examples of key findings	Refs
Pathogenesis of RA	Fibroblast-like synoviocytes in RA-affected joints have a distinct DNA methylation pattern, and express genes involved in the JAK–STAT pathway and HOX genes	77
	$CD68^+$ macrophages are central effector cells in RA	118
	$CD3^+CD45RO^+$ T_H17 cells are pivotal in RA	72
	$CD20^+CD22^+$ B cells produce antibodies and cooperate with T cells in RA	23
Synovial biomarkers of early arthritis	JNK activation is increased in early RA but not in undifferentiated arthritis	101
	Synovial CD22 and CD38 expression distinguishes patients with RA from those with non-RA disease	99
Synovial biomarkers that correlate with treatment response and disease severity	Numbers of $CD68^+$ macrophages, $CCR7^+$ T cells and $CD20^+CD22^+$ B cells correlate with treatment-induced changes in disease activity	116,121,136
	Levels of ICAM1, MMP1 and OPG correlate with treatment response	40,48,57
	Levels of S100A8, S100A9 and S100A12 correlate with the severity of joint erosion	139
	TIE2 expression is higher in erosive disease than in self-limiting disease	100
Synovial biomarkers of RA remission	Reduced numbers of $CD68^+$ macrophages are found in patients in remission	121

CCR7, CC-chemokine receptor 7; HOX, homeobox; ICAM1, intercellular adhesion molecule 1; JAK, Janus kinase; JNK, JUN N-terminal kinase; MMP1, matrix metalloproteinase 1; OPG, osteoprotegerin; RA, rheumatoid arthritis; STAT, signal transducer and activator of transcription; TIE2, tyrosine kinase with Ig and EGF homology domains 2; T_H17, T helper 17.

of current and new treatments[35–57]. Synovial tissue analysis might also provide insights into the mechanism of action of a given agent[58]. This section discusses how synovial biopsy and analysis are carried out, and summarizes the main areas in which synovial tissue analysis has proven informative.

Retrieving synovial tissue samples

Synovial tissue can be obtained by needle biopsy, arthroplasty, arthroscopic biopsy, or using ultrasound to guide the biopsy needle or grasping forceps[58] (FIG. 2). Arthroscopic biopsy enables the direct visualization of the synovium, and the operator can select an area of synovium to biopsy. By contrast, ultrasonography enables the indirect visualization of synovial thickness, and synovial vascularity can simultaneously be assessed by Doppler ultrasonography when selecting a suitable biopsy site. Although blind biopsy has been validated, arthroscopic biopsy and ultrasound-guided biopsy procedures are favoured by the majority of investigators for proof-of-concept experiments, as sampling is more specific for synovial tissue than connective tissue using these methods[52].

Arthroscopic and ultrasound-guided biopsy procedures are safe and well-tolerated. Data from 15,682 arthroscopies performed by rheumatologists revealed a complication rate of 0.9% for haemarthrosis, 0.2% for deep vein thrombosis, and 0.1% for both wound infection and joint infection[59]. These data were reproducible at other centres, and the overall complication rate was less than 0.3%. Similarly, a systematic review reported an overall major complication rate of 0.4% for ultrasound-guided biopsy procedures[60].

Synovial tissue analysis

Questions remain about the best location from which to obtain a biopsy sample within a given joint. In particular, concerns have been raised that mediators of inflammation might be differentially expressed in different parts of the same joint, particularly in the cartilage–pannus junction (CPJ) versus non-CPJ sites, which are known to behave differently[61]. However, the numbers of T cells[62,63] and plasma cells[63], and the expression levels of several MMPs[63] and granzymes[63], are reported to be similar in biopsy samples from CPJ and non-CPJ sources.

One study did find a difference for macrophages[64], but other studies did not replicate this finding[62,63]. Studies examining the optimal number of synovial tissue specimens required for reproducible research studies suggest that at least six biopsy specimens should be obtained[58,65].

Although immunohistochemical analysis of synovial tissue (FIG. 3) has a minor clinical role in the differential diagnosis of arthritis (for example, infectious, granulomatous, infiltrative diseases or crystal arthropathies), the identification of biomarkers that could be used for diagnosis or for predicting disease progression and response to treatment remains an unmet challenge. Therefore, studies of the synovium have expanded beyond immunohistochemistry to involve methods of tissue digestion, homogenization and whole-tissue culture (FIG. 4). Methods of examining synovial tissue at a molecular level include detailed omics technologies (BOX 1). For such analysis, the synovial tissue obtained from the joint is placed on saline-dampened gauze, snap-frozen in the cryoprotective optimal cutting temperature (OCT) compound or placed directly into an RNA-stablizing solution (such as RNAlater).

Synovial fluid samples are centrifuged, and a cell pellet can be isolated or separated using a Ficoll gradient to provide synovial fluid mononuclear cells. Several prognostic biomarkers of RA have been identified in synovial fluid and validated in serum samples[66]. Studies using this strategy first identified proteins that were of potential interest in the synovial fluid, and then searched for antibodies to these proteins in the plasma[67]. The approach of

Arthroplasty
Surgical reconstruction or replacement of a synovial joint.

Arthroscopic biopsy
Minimally invasive procedure to examine a synovial joint using an endoscope.

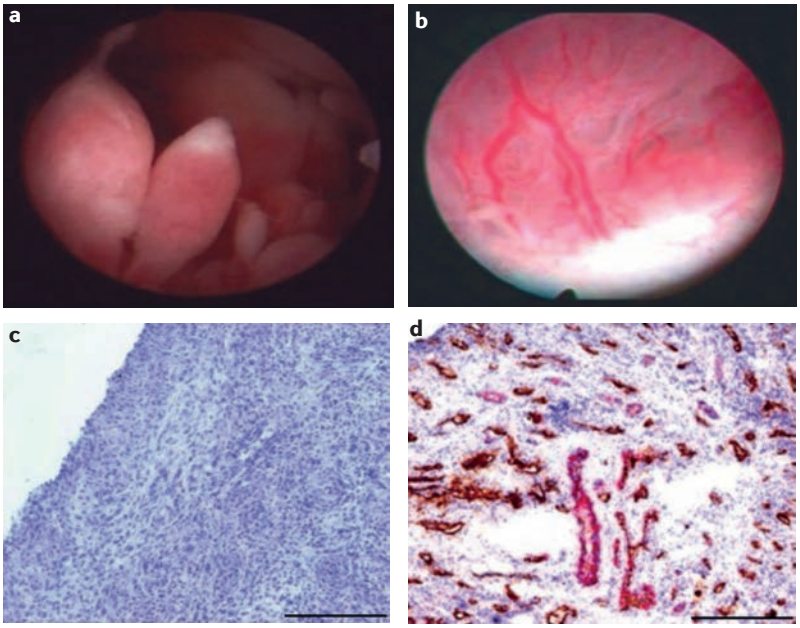

Figure 1 | **The macroscopic and microscopic appearance of rheumatoid synovial tissue.** Macroscopic images of synovial tissue from a patient with rheumatoid arthritis demonstrating inflamed and hyperplastic synovial villi (part **a**) and hypervascularity (part **b**). Representative microscopic appearance, stained with haematoxylin and eosin, demonstrating the cellular infiltrate and lining layer hyperplasia thickness (indicated by the black line) (part **c**) and representative Factor VIII immunostaining of the rheumatoid synovium demonstrating increased synovial blood vessels (part **d**) (original magnifications ×10).

obtaining and analysing different types of samples from the same patient might be useful in future experiments of synovial tissue, and the results of such research might be more easily translated into clinical practice, as serum samples can be obtained in a relatively non-invasive manner. It is important to note that although synovial fluid might reflect the synovial compartment better than does blood, it still provides only indirect information, and therefore studies of synovial tissue are essential[68]. Although most research studies of synovial tissue biopsies have involved patients with RA, which is the focus of this Review, synovial tissue sampling might also be useful in the context of other inflammatory arthropathies such as psoriatic arthritis[68–71].

Main areas of progress

As mentioned above, synovial tissue research has fuelled progress in several key areas; these areas are summarized in TABLE 1 and discussed in more detail here.

Insights into the pathogenesis of synovitis

The importance of directly analysing synovial tissue — the target tissue in RA — is evident from studies investigating the pathogenesis of RA. For example, T helper 17 (T_H17) cells are expanded in the blood of some patients with RA, and this finding provided the rationale for clinical trials of anti-IL-17 monoclonal antibodies; however, as limited T_H17 expansion occurs within the synovium of patients with RA, this therapeutic approach had little effect[72]. Indeed, studies of synovial fluid and synovial tissue from patients with RA have shown an enrichment of so-called ex-T_H17 cells at the site of inflammation[73]. This finding might explain the failure of anti-IL-17 therapy in some patients as the differentiated T cells no longer produce IL-17. Further emphasizing the importance of direct synovial tissue analysis is the fact that no circulating biomarkers have yet been identified that can provide a readout of the activity of the primary invasive cells in RA, the FLSs[74].

Synovial tissue analysis has also revealed some surprising findings regarding pathogenic mechanisms involved in RA. One study of paired biopsy samples taken from the inflamed knee joint and an inflamed small joint of patients with RA demonstrated similar mean cell numbers for all markers investigated in the synovial sublinings of both tissues[75]. Of further note, patients with clinically evident disease that manifests at small joints have been shown to have similar — albeit less pronounced — abnormalities in clinically uninvolved knee joints[64,75,76]. However, hyperplasia of the intimal lining layer seemed to depend on local processes; different joints showed no similarity in terms of the numbers of intimal macrophages or FLSs[64]. Consistent with these findings, in RA, the FLSs from different joints of the same patient show distinct DNA methylation and transcriptome signatures, as well as differences in FLS invasiveness, depending on their positional memory[77,78].

Early arthritis and disease stratification

Since 2002, cohorts of patients with early arthritis have been gathered, and have provided clinical, histological, DNA-level, mRNA-level and proteomic data; such cohorts represent instrumental resources for investigating early disease[79]. Synovial tissue analysis is beginning to have an impact on our understanding of early arthritis. Although some progress has been made in terms of diagnosing RA earlier, signs of joint destruction can already be present at the time of diagnosis and so developing our understanding of early disease is important[80]. We know today that early, aggressive treatment is more successful than is delayed treatment[81,82], and a 'window of opportunity' is suggested to exist, during which RA can be most successfully treated. Therefore, the use of biomarkers to secure a diagnosis as early as possible will enable treatment in the most timely manner and will secure the best outcomes[83]. Patients with undifferentiated arthritis might benefit most from early diagnosis. Although ACPA detection is reasonably specific (96%), the diagnostic sensitivity of ACPAs in early arthritis is 57%[81], and up to 30% of patients with RA never develop ACPAs, highlighting the need for alternative biomarkers[84,85]. An association has been defined between the presence of circulating ACPAs and the subsequent development of RA in individuals with arthralgia[86], and of bone erosions in patients with early untreated arthritis[87]. However, a positive ACPA status in those with arthralgia is associated with the subsequent development of arthritis in only 20–30% of individuals after 30 months of follow-up[88,89], further emphasizing the need for additional biomarkers.

Ex-T_H17 cells
T helper 17 (T_H17) cells can switch to become ex-T_H17 cells that no longer produce IL-17 but have the ability to produce IFNγ.

Positional memory
Cells might demonstrate different DNA 'fingerprints' depending on the site of the body at which they reside.

Undifferentiated arthritis
Inflammatory oligoarthritis or polyarthritis that does not conform to any of the recognized inflammatory arthritis types.

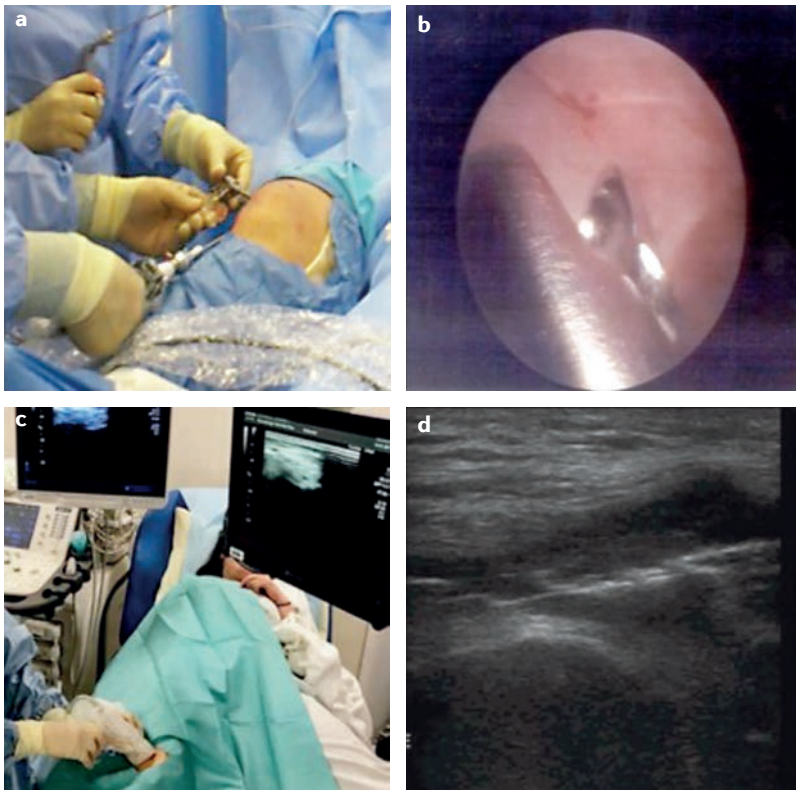

Figure 2 | **Synovial tissue retrieval methods. a** | Needle arthroscopy of the knee joint. **b** | Macroscopic image of synovial tissue biopsy using grasping forceps visualized by arthroscopy. **c** | Ultrasound-guided biopsy. **d** | Representative image of an ultrasonography scan.

A delay in diagnosing RA could arise from either a lack of a definitive biomarker or a failure to meet current diagnostic criteria, and these criteria have a considerable reliance on biomarkers; thus, further research into specific susceptibility biomarkers is warranted. Two studies published since 2015 have identified circulating biomarkers of RA in patients who lack detectable circulating ACPAs; this subset of patients is an important group to study, and data from these patients might contribute greatly to our understanding of disease pathogenesis[90,91]. Synovial tissue analysis could be key to the identification of the required biomarkers.

Cohorts of individuals who are at risk of developing arthritis have been the subject of much research. One potential corollary of such studies is the promise of a cure for RA, or a preventive approach that could detect and therapeutically target the initial breach of self-tolerance[92]. The synovial tissue of patients who are at risk of arthritis has been examined in two relatively small studies. Little evidence of synovitis was found in the first study[88], and subtle T cell infiltration was noted in the second[89]. Further study of synovial tissue samples from at-risk individuals is required, as is the analysis of other tissues, such as the lung and lymph nodes, which might be important in the very early stages of arthritis as they are the first sites at which antigen is presented[93,94].

Disease stratification
The concept that a disease can be classified into distinct subsets that exhibit differential outcomes and responses, and that can each be labelled by a biomarker or a combination of biomarkers.

The analysis of synovial tissue samples from patients with early RA has provided important insights. In initial studies, the synovia of patients with early disease have shown few molecular differences when compared with synovia of patients with late disease[23,95]. However, a study published in 2012 identified a highly expanded, specific T cell clone in the synovia of patients with early RA, which underlines the importance of T cells in early-stage disease[96]. Another study has indicated that epigenetic changes occurring in FLSs over time might define the different stages of RA after clinical onset[97]. Furthermore, in a preliminary report published in 2016, synovial tissue obtained by ultrasound-guided biopsy from unselected treatment-naive patients with early arthritis showed increased expression of the macrophage-derived chemokines CXC-chemokine ligand 4 (CXCL4; also called platelet factor 4) and CXCL7 (platelet basic protein) only during the first 3 months of symptomatic arthritis and not later in the disease[98].

In addition to identifying potential pathogenic mechanisms, synovial tissue biopsy might be useful for informing differential diagnosis in early inflammatory arthritis, as suggested by a study in which synovial CD22 and CD38 expression could distinguish patients with RA from those with non-RA disease[99]. The use of synovial biomarkers for early disease stratification was also reported in a study of 50 patients with early arthritis who had undergone synovial biopsy at inclusion and were followed for 2 years[100]. The focus was on the angiogenic processes involved in the initiation and perpetuation of synovial inflammation, in particular vascular endothelial growth factor (VEGF) and angiopoietins 1 and 2, and their tyrosine kinase receptors VEGFR and tyrosine kinase with Ig and EGF homology domains 2 (TIE2; also known as angiopoietin 1 receptor). The expression of TIE2 was significantly increased in the synovia of patients with erosive disease compared with the synovia of patients who had self-limiting disease, and the expression of activated, phosphorylated TIE-2 was significantly increased in patients with persistent non-erosive disease or persistent erosive disease compared with patients who had self-limiting disease. In addition, the activation of JUN N-terminal kinase (JNK) is elevated in the synovia of patients with early RA relative to the synovia of patients with undifferentiated arthritis, before the classification criteria for RA are met[101]. Together, these studies indicate that synovial tissue analysis can provide information relevant to disease diagnosis.

Only a limited number of studies have analysed synovial tissue from patients with early RA, and so the use of synovial tissue markers in early diagnosis is clearly still evolving. Although more research is needed, these studies suggest that a synovial biopsy at disease presentation could be a useful tool for both patients and physicians, as it could enable the stratification of early RA into short-duration, self-limiting disease (which may be erosive or non-erosive) versus severe, persistent and destructive inflammatory disease, thereby informing the most appropriate treatment strategy[102,103]. This personalized medicine approach tailors treatment on the basis of biomarkers and so-called 'disease signatures' (REF. 98), which enable

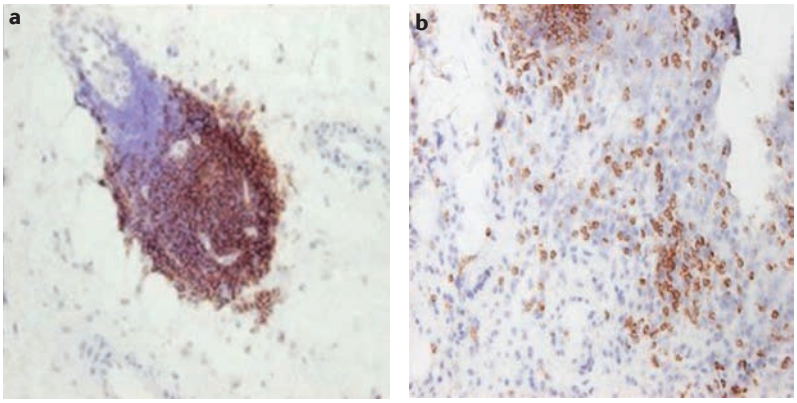

Figure 3 | **Synovial tissue immunostaining.** Representative images of biopsy-obtained rheumatoid synovial tissue demonstrating CD19$^+$ B cell lymphoid aggregate (part **a**) and CD3$^+$ T cells (part **b**). Original magnifications ×10.

disease stratification[104,105]. The sensitivity and specificity of disease stratification could theoretically be improved by using a combination of biomarkers. For example, a positive clinical response of RA to anti-TNF treatment with etanercept has been predicted using a biomarker signature comprising 13 autoantibodies and 11 cytokines. This study included three ethnically distinct populations, and for North Americans it demonstrated a positive predictive value of 71%, although independent validation is required[11].

Disease stratification is important as therapies are commonly selected on a trial-and-error basis but less than 50% of patients with RA experience a 50% improvement in their arthritis in response to any single biologic therapy[106–108]. In the time that an ineffective treatment is administered, the disease might progress, and patients could potentially experience unnecessary adverse events. Therefore, biomarkers that predict response to a given treatment will be of great clinical utility. Synovial biomarkers are likely to be of the greatest clinical utility, and a great deal of work has concentrated on studying features of the inflamed, RA-affected synovium before and after treatment. Examples of such studies, and others that have analysed the ability of synovial tissue biomarkers to predict disease prognosis and response to therapy, are summarized in the next section.

Different types of synovial biomarker

Lymphocyte aggregates. A detailed discussion of lymphocyte aggregates is beyond the scope of this Review, and the topic has recently been discussed in detail elsewhere[109]; however, we wish to briefly highlight the potential biomarker role of these structures here. A number of studies have addressed whether lymphocyte aggregates of synovitis are associated with clinical phenotype or the development of persistent, erosive disease. In two large studies, lymphocyte aggregates were found in approximately 30% of patients with established RA but did not associate with a clinical phenotype[110,111]. Similarly, the presence of lymphocyte aggregates in patients with early arthritis did not predict an aggressive disease course, and aggregates were rapidly diminished by several antirheumatic treatments[112,113]. In addition, the number of lymphocyte aggregates is reported to be predictive of the clinical response to infliximab treatment[112,114]. Positivity for lymphocyte aggregates increased the power of a prediction model that included baseline disease activity evaluated by 28-joint disease activity score (DAS28), ACPA positivity and synovial TNF expression[112]. Taken together, these studies suggest that although lymphoid aggregates may not enable the stratification of disease into subtypes, they might represent a biomarker of treatment response.

Lymphocytes. Simple cell counts (or cellular infiltrates) were recognized as RA-associated synovial tissue biomarkers more than 20 years ago. In a study published in 1989, T cell numbers were shown to decrease after at least 6 months of gold treatment, and the ratio of T_H cells to suppressor T cells or cytotoxic T cells was found to be reduced in patients who were treated successfully[35]. Furthermore, the number of biopsy samples in which B cells could be identified decreased from 36% before successful treatment to 7% after treatment[35].

Further evidence that the abundance of synovial lymphocytes (as assessed by staining for cell markers) represents a biomarker of treatment response comes from studies of the following RA therapies: 16 weeks of methotrexate, which caused a decrease in the synovial expression of markers of T cells (CD3 and CD8) and plasma cells (CD38)[115]; 4 weeks of infliximab, which reduced synovial CD3$^+$ T cell numbers in patients showing a clinical response[38]; 2 weeks of infliximab, which reduced the numbers of synovial CD3$^+$ T cells and CD22$^+$ B cells[44]; 2 weeks of prednisolone, which reduced the numbers of synovial CD4$^+$ T cells, CD5$^+$ B cells and CD38$^+$ plasma cells, as well as the number of CD55$^+$ FLSs[41]; and various durations of rituximab treatment, which partially but not invariably depleted synovial B cells, with reductions in T cells and CD68$^+$ macrophage numbers[53,116,117]. The changes in CD68$^+$ macrophage numbers after rituximab treatment have been replicated independently in another centre[118]. By contrast, one other group showed reductions in B cell numbers with minimal or no change in macrophages and T cells[119]; this variation in findings is possibly explained by differences in patient populations, methods for immunohistochemistry or analysis such as digital image analysis.

Macrophages. Although macrophages were not included in the 1989 study described at the start of the previous section[35], the most convincing evidence for a cellular biomarker of treatment response points to the macrophage marker CD68. This evidence comes from many studies, including those of patients receiving the following RA therapies: 2 weeks of prednisolone, which reduced CD68$^+$ macrophage abundance in the synovial sublining[41]; 12 weeks of gold therapy, which was associated with an abundance of changes in all synovial layers independently of the site of synovial biopsy[120]; various durations of treatment with methotrexate or gold[121], for which the reduction in CD68$^+$ macrophage numbers in the synovial sublining was particularly pronounced

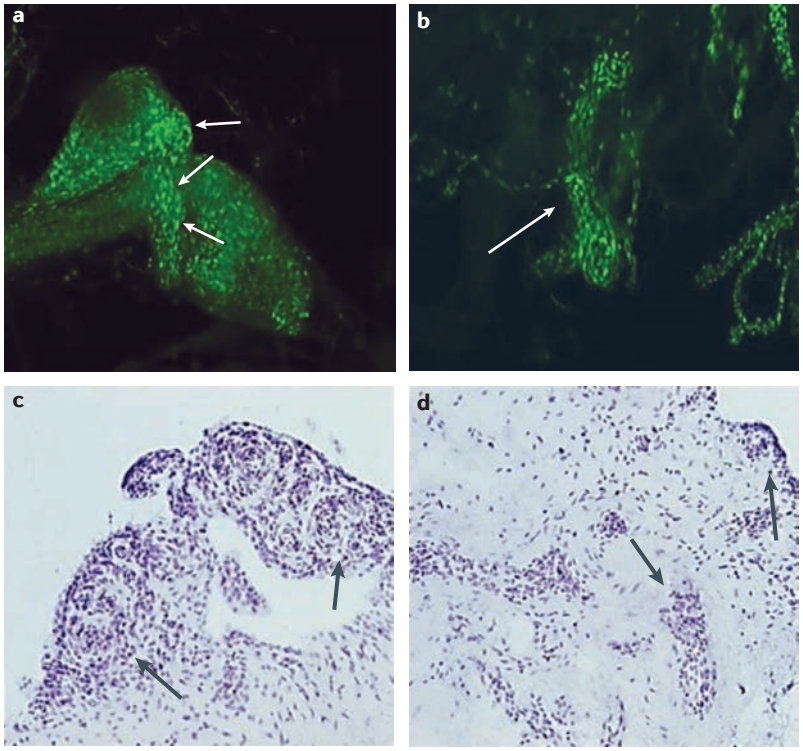

Figure 4 | *Ex vivo* **synovial tissue culture viability.** To establish *ex vivo* rheumatoid synovial biopsies, whole synovial tissue can be transferred directly from the biopsy forceps to culture medium. Synovial tissue can be maintained in culture for up to 72 h and remains viable during this time. **a** | Representative photomicrographs of synovial tissue, after 72 h in culture, stained with fluorescent calcein green indicating viable cell nuclei (white arrows). **b** | Viable blood vessels in cultured synovial tissue (white arrow). **c,d** | Representative photomicrographs of synovial tissue, after 72 h in culture, embedded in optimal cutting temperature (OCT) compound and stained with haematoxylin and eosin to demonstrate the intact structural morphology of the synovial tissue: intact lining layer, sublining layer and blood vessels (black arrows). Original magnifications ×10.

in those who showed a clinical response according to ACR criteria; 16 weeks of treatment with leflunomide or methotrexate, which were specifically associated with abundance changes in the synovial sublining and the intimal lining layer, respectively[40]; various durations of infliximab treatment, which reduced CD68$^+$ macrophage numbers in the synovial sublining[44,122]; anakinra, over 24 weeks, which reduced the size of the intimal CD68$^+$ macrophage population[42]; and various durations of rituximab treatment, which reduced the abundance of intimal lining CD68$^+$ macrophages in responders[53].

One study has systematically investigated the utility of synovial sublining-localized CD68$^+$ macrophages as a candidate biomarker across different interventions and kinetics, and found that changes in the numbers of these cells correlate with clinical improvement independently of the therapeutic strategy; the number of CD68$^+$ macrophages decreased as disease activity reduced (as measured by DAS28), thus demonstrating that such cell counts could be used as a biomarker of therapeutic response[123]. This finding was confirmed in a multicentre study that reported excellent intercentre agreement[118]. Furthermore, the sensitivity to change of synovial CD68 expression is good for both DAS28 and sublining macrophages after active treatment, including rituximab[124]; in addition, it has been shown that DAS28 is more susceptible to placebo effects than synovial CD68 expression[125]. Therefore, while we do not propose to focus on synovial biomarkers without clinical assessment, using this biomarker has been shown to be less susceptible to the placebo effect and expectation bias[123,125]. This work has led to the development of a simple decision tree to inform 'go/no-go' decision-making in drug development, which incorporates clinical assessment, mechanism of action and synovial CD68 expression and has been used in the evaluation of numerous compounds since its proposal[51]. In a ballot at the Outcome Measures in Rheumatology (OMERACT) 9 conference general assembly in 2008, 59% of the delegates agreed that CD68 expression in synovial tissue is less susceptible to a placebo effect and expectation bias than clinical evaluation, compared with 13% who disagreed[118].Therefore, substantial evidence exists to suggest that synovial CD68 expression in synovial sublining macrophages demonstrates validity, reliability and feasibility as a biomarker of disease activity and could therefore be used to assess the therapeutic efficacy of novel treatments[118,123,125,126]. All of these studies have used the same standardized techniques of immunohistochemistry, which have been extensively validated across multiple EULAR European Synovitis Study Group centres[118].

By contrast, three studies from the same centre reported minimal or no change in macrophage cells, possibly owing to the use of different methology; in studies of rituximab[119], abatacept[127] and, more recently, the signal transducer and activator of transcription (STAT) inhibitor tofacitinib[128] some reduction in sublining macrophages was apparent but this reduction was not statistically significant. A proof-of-concept study of a CC-chemokine receptor 1 (CCR1) antagonist, used at high doses to achieve high levels of receptor occupancy, did show a reduction in macrophages, CCR1$^+$ cells and a trend towards clinical response[129]. Additionally, in the single study in which similarly high levels of CCR1 receptor occupancy were achieved there was clear evidence of clinical efficacy[130], supporting the predictive value of this approach.

Cytokines. As mentioned in the 'Features of the inflamed joint' section, the increased expression of several cytokines in inflamed synovial tissue is well established. Indeed, synovial TNF and IL-6 concentrations correlate with disease activity, independently of disease duration[26].

With regards to the effects of treatment on cytokines, the expression levels of IL-1β and TNF were 40% (95% CI 18–56%) and 52% (95% CI 10–74%) lower, respectively, following prednisolone therapy compared with placebo treatment[41]. Notably, this effect was mainly attributable to changes in the synovial sublining, and seemed to correlate with clinical improvement[41]. Significant changes in cytokine expression have also been reported in the synovial lining, perivascular tissue and connective tissue after 12 weeks of gold treatment[35]. In the intimal lining layer, the levels of IL-1α, IL-1β and IL-6 were significantly reduced after treatment, and this reduction seemed to correlate

with clinical response. TNF was also reduced in all three areas, but the reduction in the synovial lining was not statistically significant[35]. In another study, TNF levels were only slightly reduced in synovial samples from patients who received 16 weeks of treatment with either methotrexate or leflunomide[40]. IL-1β levels were only moderately reduced in the leflunomide-treated patients, whereas reductions in the methotrexate-treated patients were significant, which potentially reflects the different mechanisms of action of these DMARDs[40]. Another study has also reported that the expression of IL-1β (but not that of IL-1α) is significantly reduced after 16 weeks of treatment with methotrexate and found that this reduction correlated with clinical response[115].

As highlighted above, targeting cytokine signalling pathways, for example the STAT pathway, is an interesting and novel approach. Tofacitinib has shown significant clinical benefit in patients with RA and is associated with a significant reduction in expression of phosphorylated STAT in synovial tissue, which suggests that the level of phosphorylated STAT could be a useful biomarker of response to this therapy[128]. Although not itself a cytokine, acute serum amyloid A (A-SAA) regulates the expression of cytokines and is expressed in RA synovial tissue, where it has a role in inducing angiogenesis, cell–matrix interactions, and the expression of chemokines and MMPs[131]. Furthermore, blockade of the A-SAA receptors scavenger receptor class B member 1 (SRB1)[131] and Toll-like receptor 2 (TLR2) inhibits FLS migration and invasion in synovial explants from patients with RA[132]. Importantly, baseline serum A-SAA levels independently correlate with the 28-joint swollen joint count and 1-year radiographic progression in patients with RA[20]. Therefore, serum A-SAA is a promising biomarker of disease activity, warranting further investigation of its expression in the synovia of patients with RA[133].

Chemokines. Leukocytes are attracted to target tissues by soluble chemotactic cytokines termed chemokines, which are released from activated cells in the tissue to stimulate leukocyte migration through the endothelial barrier[134]. The chemokine monocyte chemotactic protein 1 (MCP1; also known as CCL2), among others, is expressed abundantly in both serum and synovial tissue samples from patients with RA[49]. The development of clinical signs of synovial inflammation in RA is specifically associated with the increased synthesis of CXCL8 (also known as IL-8)[135], and the expression of both CXCL8 and MCP1 in synovial tissue (both the lining and sublining) reflects response to therapy in patients with active RA who have received infliximab; the synovial expression of growth-regulated protein-α (GROα), RANTES (also known as CCL5) and MIP1β (also known as CCL4) was also reduced but not to a significant extent[44]. Thus, chemokines could represent a target and a biomarker of treatment response. Indeed, a =proof-of-concept study of an oral CCR1 antagonist in patients with RA showed a trend towards clinical improvement and a concomitant, significant reduction in synovial macrophage numbers and chemokine expression, suggesting that targeting CCR1 results in changes that could also represent biomarkers of response to this antagonist[129].

S100 proteins. Similarly to some cytokines and chemokines, the S100 protein family — which comprises closely related, low-molecular-weight (9–14 kDa) acidic calcium-binding proteins — have pro-inflammatory effects, and they are overexpressed in inflammatory compartments. S100 proteins are involved in calcium-dependent cell activities such as cytoskeleton regulation, and cell migration and adhesion, and they also have extracellular roles[136]. S100A8 (also known as MRP8) and S100A9 (also known as MRP14) regulate myeloid cell function and control inflammation[137], and S100A12 (also known as MRP6) has important activities in relation to innate and acquired immune responses[138]. One study using quantitative proteomics demonstrated an association between the severity of joint erosion in RA and the levels of S100A8, S100A9 and S100A12 in both synovial fluid and serum samples[139]; this potential role of S100 proteins as synovial biomarkers requires further study.

Adhesion molecules. The expression of intercellular adhesion molecule 1 (ICAM1) is significantly reduced in patients with RA who are treated with either leflunomide or methotrexate[40]. Notably, the significant decrease in ICAM1 expression was seen only in those who responded to treatment. The expression of vascular cell adhesion molecular 1 (VCAM1) was reduced in both treatment groups, but this reduction was significant only in the leflunomide-treated patients[40].

Another study demonstrated that the expression of VCAM1 and E-selectin was significantly reduced after 16 weeks of treatment with methotrexate, but in this study the changes in ICAM1 expression did not reach statistical significance[115]. Similarly, treatment with infliximab has been shown to reduce VCAM-1 and E-selectin expression in repeat biopsies taken 4 weeks after treatment[38]. Interestingly, the effect of anakinra might be dose-dependent, as patients taking a dose of 150 mg per day — but not those receiving a lower dose of 30 mg per day — were shown to have reduced synovial expression of E-selectin, ICAM1 and VCAM1 (REF. 42). Together, these studies highlight the biomarker role of synovial adhesion molecule expression.

Mediators and products of bone, cartilage and synovial tissue degradation. Serum levels of collagen biomarkers and MMPs are known to predict radiographic progression in RA, and therefore could represent prognostic biomarkers[27,140], but could the synovial levels of these molecules also be a biomarker?

Although MMPs are present in normal synovial fluid, their concentrations are increased in synovial fluid from patients with RA, psoriatic arthritis and OA[17,141,142]. MMP1, in particular, might be a synovial biomarker of treatment response, as suggested by a study reporting that monotherapy with methotrexate or leflunomide significantly reduced the expression of MMP1 and the MMP1:TIMP1 ratio in synovial tissue samples from

patients with RA after 4 months of treatment[40]. Notably, the changes were more pronounced in patients who fulfilled the ACR20 response criteria[40].

In addition to studies of matrix-degrading MMPs, a number of studies have analysed the effects of immunomodulatory treatment on synovial mediators of bone destruction. Treatment with either infliximab or etanercept increases the expression of osteoprotegerin (OPG; also known as TNFRSF11B) in synovial tissue, but had no effect on the expression of receptor activator of nuclear factor-κB ligand (RANKL; also known as TNFSF11), resulting in an increased OPG:RANKL ratio[48]. By contrast, rituximab induces a 99% decrease in the numbers of receptor activator of nuclear factor-κB (RANK)-positive osteoclast precursors and 37% decrease in RANKL expression, but only a nonsignificant reduction in synovial OPG expression[143]. However, not all RA therapies induce changes in the levels of these bone-destructive mediators; indeed, abatacept does not significantly affect the synovial levels of mRNA expression of OPG, RANK or RANKL[127], suggesting that the biomarker role of these synovial molecules might only be relevant in specific settings.

Antigens and antibodies. The expression of antigenic proteins has been described in synovial tissue samples from patients with RA. For example, one study reported that deiminated protein — such as the α- and β-chains of fibrin — present in RA-affected synovia seem to be major antigenic targets of ACPAs[144]. In addition, anti-Sa antibodies that recognize deiminated vimentin have been isolated from RA-affected synovia and seem to be specific to RA[145]. Intracellular citrullinated proteins that colocalize with ACPA reactivity have also been identified in synovial tissue from patients with RA[146], but the presence of citrullinated antigens in synovia is not specific to RA[24]. Finally, anti-fillagrin antibodies are produced by local plasma cells that are resident in the RA pannus[147], and thus could also be synovial biomarkers of RA.

Gene-expression profiles. Most of the previous discussion has focused on protein-level data, predominantly from immunohistochemical analyses; however, as mentioned above and discussed in BOX 1, studies have also identified changes in synovial gene-expression profiles, and these profiles could represent biomarkers. An example of how transcriptomic data can be clinically useful comes from a study that used these data to create a rule-based classification that could differentiate between RA and OA[148]. In addition, gene-expression variance among patients with RA has been described for genes involved in processes such as cell proliferation, cell survival, angiogenesis and the regulation of inflammation[149].

Several studies have investigated links between gene-expression profiles and treatment response; for example, genes involved in inflammation shown to be upregulated in pretreatment biopsy-obtained synovial tissue from patients with RA who subsequently responded to anti-TNF therapy[150]. In a larger follow-up study, RNA analysis of pretreatment synovial tissue from patients with RA who were positive for lymphocyte aggregates revealed that 38 transcripts were associated with clinical response to infliximab treatment[114]. A study of paired synovial tissue samples taken from patients with RA before and 12 weeks after initiation of adalimumab treatment also identified genes that were differentially expressed in samples from responders and non-responders[151]. These genes could be split into two distinct families: genes involved in the regulation of immune responses and genes involved in the regulation of cell division. To confirm the microarray findings, the synovial expression of selected molecules was assessed using specific antibodies, and the expression of IL-7 receptor α-chain (IL-7Rα), CXCL11, IL-18, IL-18 receptor accessory protein (IL-18RAP) and the proliferation marker MKI67 was found to be significantly higher in poor responders than in moderate and good responders. Thus, these findings link gene-expression changes to protein-level changes and, consistent with studies discussed above, they emphasize the role of molecules involved in cytokine and chemokine signalling as potential biomarkers of treatment response[151].

Gene-expression analyses also support the role of macrophages and T cells as biomarkers of treatment response. For example, a study of paired synovial biopsies performed in patients with RA before and after initiation of rituximab treatment revealed that clinical responders demonstrated higher synovial expression of macrophage-associated and T cell-associated genes, whereas those with a poor clinical response showed higher synovial expression of IFNα and genes associated with matrix remodelling[152].

Challenges in biomarker identification

Most studies of serial synovial biopsies have been performed on patients with known diagnoses and have aimed to investigate response to treatment. There remains a critical need to identify diagnostic biomarkers that can be used in clinical practice. Biomarkers could reduce the time taken and patient numbers required to evaluate the potential efficacy of new drugs[51,153]. The number of patients with active disease who are eligible to participate in studies is limited. As with all trials, the number of patients who are to be put at risk by exposure to drugs at an early stage of drug development, as well as to be placed on placebo, are restricted by ethical considerations[154].

Although finding biomarkers in peripheral blood is attractive because obtaining blood samples is more feasible and less invasive than synovial biopsy, the inflamed synovium is the ultimate target of inflammation and should thus be a rich source of potential biomarkers. Furthermore, many confounding factors might interfere with peripheral blood profiles. Some authorities have suggested that a more targeted approach to searching for serum markers should first involve the identification of potential biomarkers in the inflamed synovial joint, and then the study of these biomarkers in the serum[155]. Such an approach has demonstrated clinical utility in patients with RA in a study reporting that candidate peripheral biomarkers of synovial pathotype predicted response to biologic therapy[156].

New technologies are advancing synovial tissue analysis, but several issues remain to be addressed. Although new technologies have enabled faster and more complete

analyses of proteins, the high complexity of proteins and protein isoforms in the synovial joint makes the interpretation of 'shotgun' proteomic data challenging. The interpretation of data from microarrays is also problematic; for example, three widely used microarray platforms have demonstrated poor reproducibility[157]. In addition, as array datasets contain high levels of background signals, they have decreased sensitivity to transcripts that are present in low numbers[158].

Cost and time are also important issues; for example, the development of high-quality immunoanalytical assays can be slow and expensive, which limits the verification of candidate biomarkers, despite the increase in the availability of means to biopsy synovial tissue. In addition, when data from control synovial tissue specimens is available, OA is often used as a disease control, although it is increasingly recognized that OA has an underlying inflammatory response, albeit one that is limited and less associated with specific autoimmunity than is the response that underlies RA[159]. These issues perhaps explain why although a great many genomic biomarkers exist that can predict response to treatment or who is most at risk of adverse events in many areas of medicine, rheumatology seems to have experienced only limited benefit from this emerging field. Future progress in identifying genomic biomarkers, and other types of biomarker, will probably be fuelled by synovial tissue analysis, which has not yet been extensively performed in some areas; for example, although many studies have attempted to identify gene-expression profiles that can predict response to anti-TNF treatment, to our knowledge only those reported above have used synovial tissue to search for these[112,114,151].

Conclusions

Synovial tissue represents the target tissue of autoimmune arthritides such as RA. New, safe methods of obtaining samples of synovial tissue are becoming more widely available. In this Review we have highlighted those studies that analyse the cellular and molecular characteristics of RA synovial tissue and how the results have advanced the field in terms of patient stratification, therapeutic target development and identification of biomarkers of response to therapy. In addition, we have reviewed the use of synovial tissue analysis as an outcome measure for clinical trials in RA. Finally, the future application of rapid advances in molecular technologies to synovial tissue analysis will probably lead to major benefits for patients with RA.

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Visual loss and other cranial ischaemic complications in giant cell arteritis

Alessandra Soriano[1,2], Francesco Muratore[1,3], Nicolò Pipitone[1], Luigi Boiardi[1], Luca Cimino[4] and Carlo Salvarani[1,3]

Abstract | Giant cell arteritis (GCA) is the most common form of vasculitis in individuals aged 50 years and over. GCA typically affects large and medium-sized arteries, with a predilection for the extracranial branches of the carotid artery. Patients with GCA usually present with symptoms and signs that are directly related to the artery that is affected, with or without constitutional manifestations. The most dreaded complication of GCA is visual loss, which affects about one in six patients and is typically caused by arteritis of the ophthalmic branches of the internal carotid artery. Before the advent of glucocorticoid treatment, the prevalence of visual complications was high. Increasing awareness by physicians of the symptoms of GCA and advances in diagnostic techniques over the past twenty years have also contributed to a substantial decline in the frequency of permanent visual loss. Ischaemic brain lesions are less common than visual lesions, and mostly result from vasculitis of the extradural vertebral or carotid arteries. In the case of both the eye and the brain, ischaemic damage is thought to result from arterial stenosis or occlusion that occurs secondary to the inflammatory process. The inflammatory response at the onset of arteritis, its role as a predictor of complications and the role of traditional cardiovascular risk factors have been extensively investigated in the past decade. In this Review, the epidemiology, risk factors, clinical presentation and current therapeutic approach of GCA-related ischaemic events are discussed, with a particular emphasis on visual loss.

[1]*Division of Rheumatology, Azienda Ospedaliera, Istituto di Ricovero e Cura a Carattere Scientifico di Reggio Emilia, Viale Risorgimento 80, 42100 Reggio Emilia, Italy.*
[2]*Campus Bio-Medico, University of Rome, Via Álvaro del Portillo 200, 00128 Rome, Italy.*
[3]*Università di Modena e Reggio Emilia, Via Università 4, 41121 Modena, Italy.*
[4]*Division of Ophthalmology, Azienda Ospedaliera, Istituto di Ricovero e Cura a Carattere Scientifico di Reggio Emilia, Viale Risorgimento 80, 42100 Reggio Emilia, Italy.*

Correspondence to C.S.
salvarani.carlo@asmn.re.it

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Giant cell arteritis (GCA, also known as temporal arteritis), is the most common form of vasculitis in individuals aged 50 years and over, and occurs twice as frequently in women as in men. GCA predominantly affects the aorta and its main branches, particularly the extracranial branches of the carotid arteries (BOX 1), causing a whole gamut of clinical manifestations, which differ depending on the inflamed arteries involved. Visual loss is one of the most feared cranial ischaemic complications of GCA. This clinical manifestation is typically an early event in the disease course, and is usually a consequence of arteritis of the posterior ciliary arteries[1] (BOX 1); less frequently, GCA-related loss of vision is secondary to arteritis of the central retinal artery, posterior optic neuropathy or ischaemia of the visual cortex. Overall, visual loss occurs in about one in every six patients with GCA[2,3]. Visual loss caused by GCA can be partial or complete, and can affect one or both eyes; transient visual loss often develops into permanent sight loss if treatment with glucocorticoids is not commenced swiftly[4]. Glucocorticoids can usually prevent further sight loss within a few days of the onset of treatment, but can rarely revert established blindness[4].

Prompt recognition of GCA is therefore key to preserving intact vision. Temporal artery biopsy (TAB) remains the gold standard for securing the diagnosis of GCA, but imaging techniques, especially colour Doppler ultrasonography, have an increasingly important role in detecting signs of arteritis[5]. However, even when TAB is correctly performed, the biopsy might still be negative (biopsy-negative GCA) because of a lack of involvement of the temporal arteries or due to random sampling of a non-inflamed arterial segment.

In this Review article, we will summarize what is known regarding the epidemiology of severe cranial ischemic complications in GCA and its risk factors, discuss the mechanisms underlying visual loss, delineate the diagnostic approach for patients with GCA and outline the current therapeutic approach to GCA-related ischaemic events, with a particular emphasis on visual loss.

Key points

- Visual loss is the most feared manifestation of giant cell arteritis (GCA) and occurs in up to 20% of patients before glucocorticoid therapy is commenced
- Anterior ischaemic optic neuropathy (AION) owing to arteritis of the posterior ciliary arteries is the most common cause of visual loss in GCA and must be differentiated from non-arteritic AION
- Cerebrovascular accidents — stroke and transient ischaemic attack — occur in 1.5–7% of patients with GCA and are caused by stenosis or occlusion of the extradural vertebral or carotid arteries
- A previous ischaemic event in GCA is the strongest predictor for a subsequent event; patients with traditional cardiovascular risk factors and a lower inflammatory response are more likely to develop ischaemic manifestations
- Adequate doses of glucocorticoids in GCA largely prevent further cranial ischaemic events, but are scarcely effective at improving established visual loss
- Fast-track clinics for the diagnosis of GCA might substantially reduce the occurrence of permanent sight loss by reducing diagnostic delay.

Epidemiology studies

Ocular ischaemic events. Visual loss in GCA was first reported by Jennings in 1938, who described the left optic disc of a 66-year-old lady, who developed complete blindness in her left eye, as being pale and the retinal artery as being occluded on fundoscopy[6]. Sight loss is one of the leading causes of morbidity related to GCA. Notably, studies conducted before the advent of glucocorticoid treatment showed a high prevalence (35–60%) of visual complications[7–9], whereas those performed after glucocorticoids became available have revealed much lower rates of visual loss (about one in six patients)[10] (TABLE 1). High rates of visual loss (22–49%) have been documented in studies carried out in ophthalmology settings[11,12], probably as a result of referral bias. Studies from different countries report similar prevalence rates of visual loss, which indicates that genetic and environmental factors do not influence the expression of GCA-related visual manifestations[2,13–15]. By contrast, some studies have noted a trend for a decreased incidence of visual complications since the middle of the last century[16,17], suggesting a favourable effect of earlier diagnosis, prompter implementation of glucocorticoid therapy, or both, on visual symptoms[16].

As a rule, ocular ischaemic events are generally considered to be associated with GCA if they occur concomitantly with GCA or up to 4 weeks after the onset of glucocorticoid therapy[12,13,15,18]. Most studies that have investigated visual lesions in GCA are retrospective in design and contain patients who have had a positive TAB, fulfilled the ACR classification criteria for GCA, or both[2,9,12,13] (TABLE 1). These inclusion criteria probably overestimate the overall prevalence of GCA-related cranial ischaemic events, as they do not adequately include patients who mainly present with large-vessel vasculitis, a subset less prone to developing ischaemic lesions[19]. From these studies, visual loss of varying severity was the most common complication in patients with GCA, followed by amaurosis fugax and diplopia, whereas eye pain was extremely rare[12,16]. In the vast majority of cases, visual loss was an early event, which occurred before, or within a few days of, the onset of glucocorticoid treatment[10,20].

Cerebrovascular events. Cerebrovascular accidents (CVAs) — namely stroke and transient ischaemic attack (TIA) — are serious but rare complications of GCA, occurring in 1.5–7% of patients in studies that have limited the inclusion of such events to those occurring concomitantly with GCA up to 4 weeks after the onset of glucocorticoid therapy[2,13,15,21–25] (TABLE 2). In one of the largest population-based studies, the prevalence was 2.8% (8 out of 287 cases)[22]. The same rate was also found in a series of 180 patients with biopsy-proven GCA from northern Italy[15]. Higher prevalence rates were reported when the time frame after the onset of glucocorticoid therapy was extended beyond 4 weeks[26]. However, even in long-term observational studies, the association was strongest in the first month after the diagnosis of GCA[26], which is consistent with the notion that GCA-related ischaemic events occur most frequently before or shortly after the institution of glucocorticoid therapy[13]. Overall, stroke has been documented more often than TIAs[27]. A similar prevalence of CVAs has been reported in studies from different countries, suggesting a negligible role for genetic and environmental factors in the expression of GCA-related brain ischaemic events[13,21,22].

Clinical aspects and assessment

Ocular ischaemic events. Visual loss in GCA is usually painless and sudden in onset; monocular vision loss occurs most often, but binocular involvement, either from the onset of vision loss or developing after monocular ischaemia, has also been well documented[2,10,20,21]. Patients typically report a feeling of shade covering one eye, which can progress to total blindness. GCA-related visual loss is usually an early event, appearing before diagnosis (normally occurring before or within 1 week of the onset of glucocorticoid therapy), and is irreversible, although glucocorticoids can prevent further ocular ischaemic events within a few days of administration[11,28]. Amaurosis fugax is reported in 10–15% of patients and can precede permanent visual loss[12,28].

Anterior ischaemic optic neuropathy (AION) is by far the most common cause of visual loss in patients with GCA (occurring in about 80% or more of cases), followed by central retinal artery occlusion (5–15% of cases). Diplopia is reported in ≤10% of patients and is characteristically transient[28]. Diplopia is thought to result from extra-ocular muscle or cranial nerve ischaemia[29] and might precede permanent visual loss. Posterior ischaemic optic neuropathy and cortical blindness are distinctively rare (≤3% of patients with GCA have either condition)[2,18,30,31]. AION is usually caused by occlusive arteritis of the posterior ciliary arteries (BOX 1). Cilioretinal artery occlusion has also been demonstrated, but is nearly always found in association with occlusion of the posterior ciliary arteries[28]. A combination of more than one lesion has also been described in a few patients[2,18].

The optic disc of patients with AION shows slight pallor and oedema with small haemorrhages on early fundoscopy[12] (FIG. 1a). After a few weeks, optic atrophy develops (FIG. 1b). In patients with posterior ischaemic optic neuropathy, although the fundus shows no abnormality on

Fundoscopy
A routine examination (also known as ophthalmoscopy) for looking at the back of the eye (fundus)

Amaurosis fugax
Visual loss in one or both eyes that is transient and painless.

Diplopia
Double vision.

Cortical blindness
Blindness resulting from ischaemia of the visual cortex.

Stenosis
Abnormal narrowing of a blood vessel.

Vasa vasorum
A network of small blood vessels that supply the walls of blood vessels

fundoscopy, optic atrophy develops 6–8 weeks later[28]. No abnormalities of ocular structures have been observed in the rare cases of cortical blindness[12].

Cerebrovascular events. GCA-related CVAs usually occur within one month of the diagnosis of GCA, and can be prevented by initiating glucocorticoid therapy[28]. CVAs can also be the first clinical manifestation of GCA: in a study of 98 patients with GCA complicated by CVAs, CVAs represented the initial presentation in 5 out of 68 biopsy-proven cases[24].

In the overwhelming majority of cases, cerebrovascular events are the result of stenosis or occlusion of the extradural vertebral or carotid arteries[22] (BOX 1). Overall, 40–60% of GCA-related strokes involve the vertebrobasilar system, compared with 15–20% in the case of strokes caused by atherosclerosis[24,32]. Colour-Doppler ultrasonography reveals carotid and/or vertebral stenoses or occlusions variably associated with hypoechoic mural thickening of the proximal segments[33].

Involvement of the intracranial arteries is exceptional, probably because GCA tends to affect arteries with elastic tissue in their wall, and intradural arteries contain little or no elastic tissue[34]. An additional reason for the rarity of intracranial arteritis in GCA might be the absence of vasa vasorum, through which inflammatory cells enter the vessel wall, from the intracranial arteries[35]. Patients with intracranial arteritis represent a subset of those with GCA with a fatal disease course that usually fails to respond to glucocorticoids. MRI typically reveals brain ischaemic lesions, whereas magnetic resonance angiography or conventional angiography shows stenoses or occlusions of large intracranial vessels in these patients[35] (FIG. 2).

Differential diagnosis. The main differential diagnosis of sudden-onset, painless loss of vision is non-arteritic anterior ischaemic optic neuropathy (NAION). NAION is caused by a non-thrombotic lesion of the optic nerve head, probably as a result of a drop in blood pressure, which occurs physiologically at night and can be aggravated by

Box 1 | **The blood supply to the eye and brain**

Blood is supplied to the eyes and brain by branches of the internal carotid arteries and vertebral arteries. The aorta first delivers blood to the common carotid and subclavian arteries. The common carotid arteries then divide into the external and internal carotid arteries. The internal carotid arteries enter the skull to deliver blood to the brain via cerebral arteries; the ophthalmic arteries branch off from the internal carotid arteries and pass through the optic canal to enter the orbit, dividing into numerous branches including the central retinal artery, the posterior ciliary arteries and the anterior ciliary arteries. The short posterior ciliary arteries supply part of the choroid and ciliary processes, whereas the long posterior ciliary arteries supply the iris, the choroid, and the ciliary body.

The optic nerve can be divided into an anterior (optic nerve head) and a posterior part. The main source of blood supply to the optic nerve head are the posterior ciliary arteries. The surface nerve fibre layer is mostly supplied by the retinal arterioles arising from the central retinal artery. The cilioreoretinal artery (branch of the short posterior ciliary arteries), when present, usually supplies the corresponding sector of the retinal surface layer.

This figure was adapted from Hayreh, S. S. *Am. Acad. Ophthalmol. Otolaryngol.* **78**, 240–254 (1974)[87].

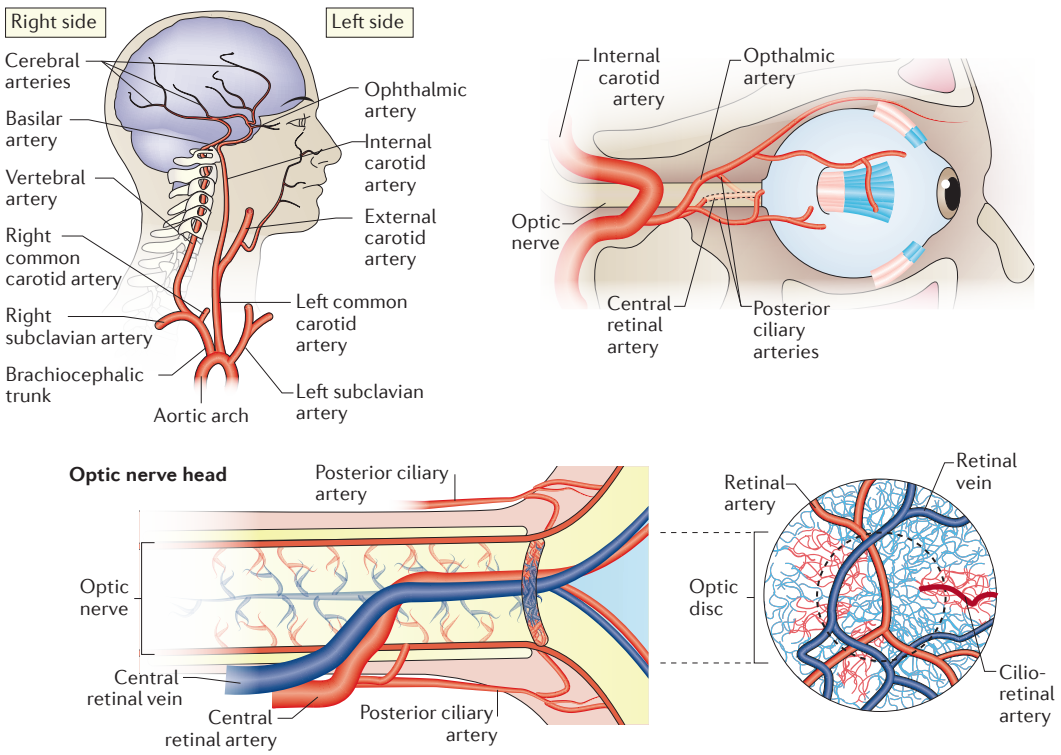

Table 1 | **Prevalence of permanent visual loss in clinical studies**

Study	Patients (n)	Diagnostic criteria	Study setting	Study type/period	Prevalence
Cooke *et al.* (1946)[9]	185	TAB+	Eye institute, USA	Retrospective	22%
Cid *et al.* (1998)[2]	200	TAB+	Three hospitals, Barcelona, Spain	Retrospective, 16 years	14%
Gonzalez-Gay *et al.* (1998)[20]	239	TAB+	Three hospitals, northern Spain	Retrospective (1975–1996)	14%
Hayreh *et al.* (1998)[12]	170	TAB+	Ophthalmology Department, USA	Prospective (1973–1995)	49%
Gonzalez-Gay *et al.* (2000)[30]	161	TAB+	Lugo Hospital, Spain	Retrospective (1981–1998)	15%
Haugeberg *et al.* (2000)[3]	53	ACR	Vest Adger County, Norway	Retrospective (1992–1996)	8%
Nesher *et al.* (2004)[13]	175	TAB+ or ACR	Four hospitals, Israel	Retrospective (1980–2000)	18%
Berger *et al.* (2009)[21]	85	TAB+ or ACR	Department of Internal Medicine, Basel, Switzerland	Retrospective (2003–2007)	32%
Salvarani *et al.* (2009)[15]	180	TAB+	Rheumatology Department, Reggio Emilia, Italy	Retrospective (1986–2005)	18%
Liozon *et al.* (2016)[18]	339	TAB+*	Department of Internal Medicine, Limoges, France	Retrospective (1976–2015)	16%
Saleh *et al.* (2016)[41]	840	TAB+	Skåne County, Sweden	Retrospective (1997–2010)	2%
Chen *et al.* (2016)[88]	245	ACR	Mayo Clinic (Rheumatology), USA	Retrospective (1950–2009)	8%

Abbreviations: TAB+, positive temporal artery biopsy result. *Positive arterial biopsy result (not exclusively of the temporal artery)

the use of potent anti-hypertensive medications. NAION is much more common than arteritic AION (90% versus 10% of ischaemic optic neuropathy) but ischaemic damage is less severe in NAION than in AION[29].

The differential diagnosis between arteritic AION and NAION is particularly challenging in patients with so-called occult GCA — GCA that affects the eyes only. The reported incidence of occult GCA varies according to different studies and modalities of diagnosis. Hayreh *et al.*[12] reported a 21% incidence of occult GCA in a prospective study of 85 patients seen in an ophthalmology department, with histopathological confirmation of the disease in all patients. In this context, elevated inflammatory markers point to GCA and away from NAION. Fundoscopy can also aid in differentiating arteritic AION from NAION. In NAION, fundoscopy usually reveals hyperaemic (less often, pale) oedema of the optic disc, with a small cup and consequent crowding of the optic nerve fibres[36]. Cotton wool spots and retinal infarcts are very rare in NAION, in contrast to AION, and the cup does not show any modification once oedema resolves, whereas it usually appears enlarged in AION.

Risk factors for ischaemic events

Several studies have addressed the role of risk factors in the pathogenesis of GCA-related ischaemic events.

Previous cranial ischaemic events and vessel origin. As a rule, a previous cranial ischaemic event is considered one of the strongest predictors for a subsequent event[22,30,37,38]. Along this line, jaw claudication, which is thought to be ischaemic in origin, can also predict subsequent ocular ischaemic lesions[16,21,28]. Compared to patients with cranial GCA, those with large-vessel GCA, who present less often with cranial symptoms including jaw claudication, have a reduced frequency of visual loss (4% versus 11%)[39].

Hypertension, smoking, previous ischaemic heart disease and atherosclerosis. Since the early 2000s, the presence of hypertension before the onset of GCA has been reported as a risk factor for severe ischaemic complications in patients with biopsy-proven GCA, and has subsequently been confirmed in some, but not all, studies[1,40]. Some investigators have also reported a positive association between the use of anti-hypertensive agents (notably beta blockers) and ischaemic lesions[41], but this association might be the result of a confounding effect by indication.

In addition to hypertension, smoking and a past history of ischaemic heart disease have been associated with the occurrence of stroke in GCA[15,22]. In an Italian population-based cohort study, hypertension, previous ischaemic heart disease and low levels of inflammation were associated with a higher risk of the occurrence of ischaemic events in GCA[15]. Another study found a positive association between traditional risk factors for atherosclerosis and GCA-related ischaemic events, suggesting that patients with atherosclerosis might be unable to efficiently mount appropriate angiogenic compensatory mechanisms[40].

Inflammation and inflammatory markers. Moderate, but not excessively high, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels at the time of diagnosis are predictors of irreversible visual loss[1,40]. By contrast, markedly elevated ESR and CRP, as well as anaemia, are linked to a decreased risk of ischaemic events[15,37,40]. A population-based study showed that patients with anaemia and an ESR value of >100 mm/h at the time of diagnosis had fewer severe ischaemic events compared with those without anaemia and with lower ESR levels; however, in the multivariate analysis, only anaemia negatively predicted the risk of severe ischaemic manifestations[42].

Cotton wool spots
An abnormal manifestation where fluffy white patches are observed on the retina during fundoscopy examination.

Jaw claudication
Pain in the jaw, particularly when talking or eating

Similarly, the presence of systemic inflammatory manifestations (driven by proinflammatory cytokines, which also induce acute-phase reactants) is a protective factor against ischaemic lesions[15,37,38,40]. A strong inflammatory response could protect against ischaemic lesions by two means: first, patients with pronounced inflammatory features might be seen sooner in the clinic and thus receive more prompt treatment, and, second, IL-6, a key proinflammatory cytokine, is endowed with angiogenic properties, and might therefore counteract arteritis-induced ischaemia. An explanation for why a strong inflammatory response could be protective against GCA-related ischaemic events has been put forward by Cid and colleagues[43]. These researchers demonstrated that serum angiogenic activity and tissue neovascularization in sections of the temporal arteries were most prominent in patients with GCA with a strong acute phase response and in those without ischaemic events, and that patients with GCA who have ischaemic complications have lower tissue expression and circulating levels of IL-6 than those patients with no ischaemic events[43,44]. The angiogenic activity of IL-6 might therefore confer a potential protective mechanism against ischaemia in GCA.

Genetic associations. Associations between genetic variations, such as the presence of the *HLADRB1*04* allele, and visual complications[45] have been described, and the strong contribution of HLA class II molecules to susceptibility to GCA has been confirmed through a large-scale genetic analysis[46].

A study from northern Italy reported an association between AION and homozygosity for the *PLA2* allele of the *PLA1/A2* polymorphism of *ITGB3* [47]. Given that the *PLA2* allele is linked to increased platelet adhesion and aggregation, this finding points to a potential role for thrombosis in inducing cranial ischaemic complications in GCA[48]. Studies from Spain have reported associations between ischaemic events and the presence of the *A2* allele in the 5′ regulatory region at position 1273 of *CRH*, the G variant of the 634 C/G polymorphism in *VEGFA*, the T variant of the *CD40 rs1883832* C/T polymorphism, and the CA repeat polymorphism in the first intron of the allele of *IFNG*[45,49–51]. In addition, patients with visual ischaemic complications express high amounts of *IFNG* mRNA[51], indicating that an interferon signature might contribute to a response-to-injury reaction at the vessel level and subsequent luminal obstruction, at least in a subset of patients.

Histology. With regards to histological findings, moderate to severe intimal hyperplasia and the presence of giant cells on histology correlated with cranial ischaemic events[52,53] and with permanent visual loss[54], respectively. Permanent visual loss is associated with the presence of calcification in the temporal artery, a sign of atherosclerosis, suggesting that atherosclerosis and GCA might share some inflammatory pathways[55]. By contrast, although anticardiolipin antibodies are found more frequently in patients with GCA than in healthy individuals, there is no association between anticardiolipin antibodies and ischaemic manifestations in GCA[56]. Similarly, the incidence of severe ischaemic events in patients with GCA does not correlate with thrombocytosis[21]. In one study from Spain, patients with GCA who had a negative biopsy result (but fulfilled the ACR 1990 criteria) had less severe ischaemic complications than patients with biopsy-proven GCA[57]. However, a similar frequency of visual loss has been observed among Italian patients with biopsy-positive and biopsy-negative GCA (18.3% versus 15.8%)[15,58] although these studies were not specifically designed to correlate GCA-related visual loss with TAB findings.

Treatment

Glucocorticoids

Glucocorticoids are the treatment of choice for GCA — adequate doses quickly suppress clinical manifestations of this disorder and prevent most additional cranial ischaemic events[4] (BOX 2).

Table 2 | **Prevalence of stroke in clinical studies**

Study	Patients (n)	Diagnostic criteria	Study setting	Study type/period	Prevalence
Cid *et al.* (1998)[2]	200	TAB$^+$	Three hospitals, Spain	Retrospective 16 years	2%
Nesher *et al.* (2004)[13]	175	TAB$^+$ or ACR	Four hospitals, Israel	Retrospective (1980–2000)	3%
Ray *et al.* (2005)[89]	1141	Hospital discharge diagnosis	Ontario, Canada	Retrospective (1995–2002)	0.5%
Berger *et al.* (2009)[21]	85	ACR or TAB$^+$	Department of Internal Medicine, Basel, Switzerland	Retrospective (2003–2007)	2%
Gonzalez-Gay *et al.* (2009)[22]	287	TAB$^+$	Lugo Hospital, Spain	Retrospective (1981–2008)	3%
Salvarani *et al.* (2009)[15]	180	TAB$^+$	Rheumatology Department, Reggio Emilia, Italy	Retrospective (1986–2005)	11%
Zenone *et al.* (2013)[24]	98	ACR	Department of Internal Medicine, Valence, France	Retrospective (1999–2012)	6%
Tomasson *et al.* (2014)[26]	3408	Hospital discharge diagnosis	United Kingdom	Retrospective cohort study (1990–2010)	11%
Samson *et al.* (2015)[23]	57	TAB$^+$	Residents of Dijon, France	Retrospective (2001–2012)	7%
Lo Gullo *et al.* (2016)[27]	244	ACR	Mayo Clinic, USA	Retrospective (1950–2009)	10%

Abbreviations: TAB$^+$, positive temporal artery biopsy result.

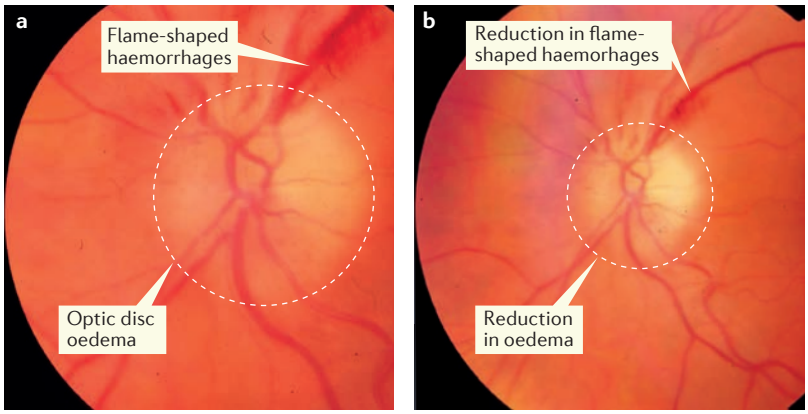

Figure 1 | **Visual loss in a patient with GCA owing to anterior ischaemic optic neuropathy. a** | In the early acute phase, fundus photography shows optic disc oedema and flame-shaped haemorrhages (20 degrees, higher magnification). **b** | After 15 days of prednisone therapy, optic disc atrophy occurs with a reduction in oedema and the size and number of flame-shaped haemorrhages (35 degrees).

The importance of prompt treatment. Prompt treatment with 40–60 mg daily of prednisone (or its equivalent) should be prescribed to all patients with suspected GCA and, although TAB should be carried out to diagnose GCA with a high degree of certainty, no difference in the sensitivity of TAB in diagnosing GCA was reported until 2–4 weeks after starting glucocorticoid treatment[59]. Patients with AION can also be prescribed oral prednisone when the likelihood of GCA is low but the result of the TAB is awaited[59–61].

Patients who present with or who are at a high risk of developing cranial ischaemic events might require a higher initial dosage of glucocorticoids, which should be administered rapidly: either 1 mg/kg/day of oral prednisone or 500–1,000 mg/day of intravenous methylprednisolone daily for 3 days (followed by oral prednisone at 1 mg/kg/day)[62–64]. The timing of glucocorticoid therapy is, in fact, probably more important than the dose and route of administration. If untreated, the second eye is likely to become affected within 1–14 days in ≤30% of patients[12].

In GCA, the initial dose of oral prednisone (or its equivalent) is usually given for 2–4 weeks until all reversible signs and symptoms have resolved and the levels of acute phase reactants are back to normal. The dose can then be gradually reduced each week, or every 2 weeks, by a maximum of 10% of the total daily dose if no flare occurs[4]. Administration of glucocorticoids on an alternate-day basis is associated with a higher rate of treatment failure compared with daily administration, and is therefore not recommended[65].

Fast-track clinics for the diagnosis of GCA might help to identify patients sooner and therefore initiate adequate treatment before complications such as blindness occur. In this regard, two studies — one from the UK[66] and one from Norway[67] — have reported that cases of permanent sight loss were decreased with a fast-track GCA pathway. A reduction in delayed diagnosis as well as a higher standard of education among general practicioners might be just two of many reasons for this outcome[66].

Oral and intravenous glucocorticoids. Two randomized controlled trials evaluated the glucocorticoid-sparing effect of an initial intravenous pulse of methylprednisolone in the treatment of GCA; however, patients presenting with cranial ischaemic events were excluded from both studies. One of these trials showed that intravenous methylprednisolone pulse therapy had no glucocorticoid-sparing effects[68], whereas the other demonstrated that pulse therapy allowed for more rapid tapering of oral glucocorticoids and helped to maintain remission[69]. Different retrospective and prospective observational studies have failed to demonstrate the superiority of an intravenous pulse over oral glucocorticoids in preventing visual complications or improving established visual loss[11,20,70–72].

The development of visual loss is extremely rare after the prompt initiation of high-dose glucocorticoids (either orally or intravenously), but pre-existing vision loss progresses in 9–27% of patients, usually within the first 6 days of treatment[10,11,20,70,73–75]. Beyond this time, visual function tends to stabilize. No difference, in terms of development of new visual loss or progression of pre-existing visual loss, seems to exist between patients treated with intravenous pulse and those treated with oral glucocorticoids[10,11,20,70,73–75]. In the only retrospective, population-based study characterizing visual prognosis in GCA, 245 patients were investigated, the majority of which were treated with oral glucocorticoids and 34 (14%) of whom permanently lost vision because of GCA. The 5-year probability of new loss of vision was 1%, whereas the probability for progression of pre-existing vision loss was 13%[10].

Improvement of visual acuity after the initiation of high-dose glucocorticoids (either orally or intravenously) occured in 4–34% of eyes, generally when treatments started early (within 48–72 hours of the onset of visual loss)[10,11,20,72,73,75–77]. However, improvement in visual acuity without a corresponding improvement in the central visual field might just reflect an increased ability to see better by eccentric fixation and not genuine visual improvement[78]. Accordingly, improvement in both visual acuity (≥2 lines) and central visual field has been reported much less frequently (0–5% of eyes)[72,73,77]. No difference in terms of visual outcome was seen between patients who received intravenous pulse and those who received oral glucocorticoids[10,11,72]. In the only study that demonstrated a superiority of intravenous pulse over oral glucocorticoids, the criterion used to determine visual recovery was the improvement of visual acuity (at least one line) — central visual field assessment was not performed[75]. Consistent with results from the aforementioned studies, the timing of treatment is probably critical. A retrospective multicentre study of 29 patients with GCA with permanent visual loss is the only study that used a cut-off interval time of 24 hours from diagnosis to treatment onset to define early treatment. After adjustment by logistic regression analysis for therapeutic regimen (intravenous pulse versus oral prednisone), early treatment was the only significant predictor of improvement (OR 17.7); the therapeutic regimen did not influence visual outcome[20].

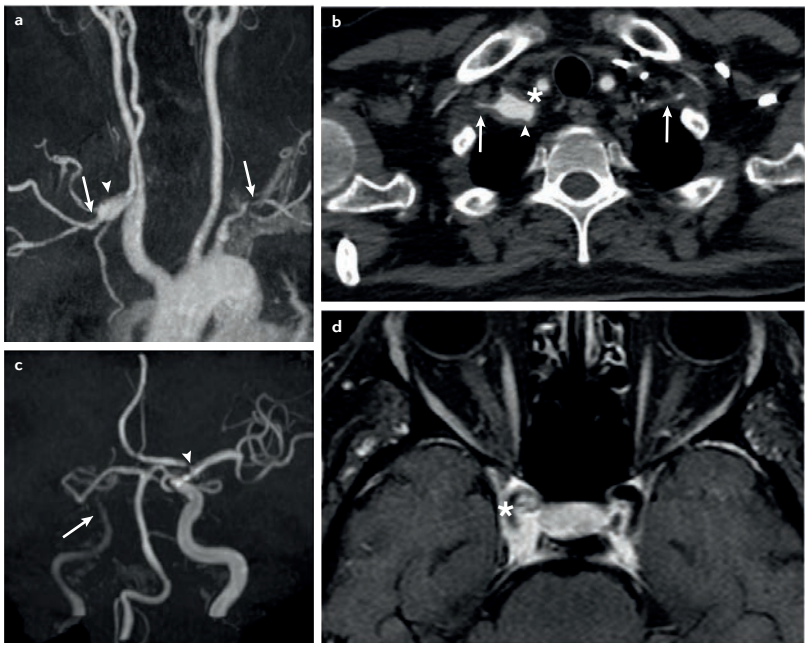

Figure 2 | **Intracranial and extracranial large vessel involvement secondary to giant cell arteritis. a** | Magnetic resonance angiography showing stenosis of the subclavian arteries (arrows) and dilatation of the origin of the right subclavian artery (arrow head). **b** | Computed tomography angiography axial view image showing arterial wall thickening of the right subclavian and common carotid arteries (asterisk), stenosis of the subclavian arteries (arrows) and dilatation of the origin of the right subclavian artery (arrow head). **c** | Intracranial magnetic resonance angiography showing stenosis of the origin of the left anterior cerebral artery (arrow), narrowing and irregularities of the right internal carotid artery (arrow head), absent flow of the A1 segment of the right anterior cerebral artery, of the right middle cerebral artery and of the left vertebral artery. **d** | T1-weighted spectral presaturation with inversion recovery (SPIR) contrast-enhanced MRI showing thickening of the vessel wall and intramural contrast enhancement of the right carotid siphon (asterisk). Image provided courtesy of Dr Lucia Spaggiari and Dr Manuela Napoli, Radiology Department, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy

Emerging treatment options

Tocilizumab. Two randomized placebo-controlled trials showed the efficacy of tocilizumab, a humanized monoclonal antibody that targets the IL-6 receptor, in the induction and maintenance of remission in patients with GCA[79,80]. In both studies, glucocorticoid treatment could be rapidly tapered and discontinued after the initiation of tocilizumab treatment — by 26 weeks in the GiACTA trial and by 36 weeks in the Swiss trial.

In the Swiss study[79], relapse-free survival was achieved at week 52 in 85% of patients in the tocilizumab group and in 20% of patients in the placebo group ($P = 0.001$)[79]. In the GiACTA trial, sustained remission at week 52 was achieved in 56% of patients treated with tocilizumab weekly and in 53.1% of patients treated with tocilizumab every other week, compared with 14% and 17.6% of patients receiving placebo and a 26-week or 52-week glucocorticoid tapering course, respectively (all comparisons $P < 0.001$)[80]. Consequently, a substantial reduction in the number of relapsing patients, number of flares, duration of glucocorticoid treatment and cumulative glucocorticoid dosage was observed in patients treated with tocilizumab[79,80]. Regardless of the treatment arm, no patients in the GiACTA trial experienced severe cranial ischaemic events, such as visual loss[80].

The GiACTA trial showed that a 26-week and a 52-week glucocorticoid taper, in combination with careful control of disease activity during the follow-up, were equally effective in preventing ischaemic complications in most patients with GCA[80]. The addition of tocilizumab to prednisone did not seem to further improve this outcome. To date, no newly-diagnosed patients with GCA who are naive to glucocorticoid treatment have received tocilizumab monotherapy, which raises the question of whether tocilizumab is effective in preventing GCA-related ischaemic events.

In a multicentre randomized placebo-controlled trial that evaluated the efficacy of adjuvant methotrexate treatment for GCA, 13.8% of patients experienced new visual loss after 1 year of follow-up, with no difference between methotrexate and placebo groups[81]. In this study, all patients received prednisone 1 mg/kg/day (not exceeding 60 mg) for 4 weeks. After 4 weeks, prednisone dosage was converted from a daily to an alternate-day regimen and reduced until discontinuation by 6 months. The higher rate of new vision loss reported in the methotrexate trial compared with the GiACTA trial is probably related to the glucocorticoid regimen that was followed in the methotrexate trial, which involved a combination of an accelerated reduction of glucocorticoid treatment plus an alternate-day taper.

Other therapies. Several retrospective studies have evaluated the effect of low-dose aspirin treatment on the risk of cranial ischaemic events in patients with newly-diagnosed GCA, with conflicting results[13,15,21,40,60,82]. In these studies, aspirin treatment was initiated in the majority of patients as an anti-platelet therapy before GCA diagnosis, most often because of pre-existing cardiovascular risk conditions. Only two studies included patients who started anti-platelet therapy subsequent to the diagnosis of GCA. However, in neither of these studies was the risk of cranial ischaemic events in patients who began low-dose aspirin treatment after being diagnosed with GCA compared with the risk in patients who were never treated[13,40]. Two meta-analyses of the data from the aforementioned studies concluded that the benefit of initiating anti-platelet therapy at the time of GCA diagnosis remains unclear or that there might only be a marginal benefit when used together with high-dose glucocorticoid treatment in patients who have established GCA without an associated bleeding risk[83,84]. A Cochrane review on the same topic concluded that there are currently insufficient data to make a definitive statement on whether aspirin is of benefit in GCA[85]. Prospective studies are needed to define the role of aspirin in GCA. Currently, the use of low-dose aspirin should follow the current recommendations for preventing complications of atherosclerosis[64].

To date, there are no data on the effect of traditional and/or biologic immunosuppressive agents in the prevention or treatment of GCA-related cranial

Box 2 | **Treating patients with GCA presenting with cranial ischaemic events**

Recommended treatment

Patients with anterior ischaemic optic neuropathy with a low index of suspicion but awaiting the temporal artery biopsy result

- Start oral prednisone 1 mg/kg/day

Patients at high risk of cranial ischaemic events (history of amaurosis fugax or unilateral visual loss)

- Immediately start 500–1,000 mg/day of intravenous methylprednisolone for 3 days followed by oral prednisone 1 mg/kg/day
- Start oral prednisone 1 mg/kg/day if intravenous pulse cannot be rapidly initiated

Patients with established bilateral visual loss

- Start oral prednisone 1 mg/kg/day

Patients at risk of cranial ischaemic events (jaw claudication)

- Start oral prednisone 1 mg/kg/day

Patients with cerebrovascular accidents (stroke and transient ischaemic attack)

- Start oral prednisone 1 mg/kg/day

Patients with intracranial vasculitis secondary to giant cell arteritis

- Start oral prednisone 1 mg/kg/day (consider intravenous pulse of methylprednisolone 1,000 mg/day for 3 days)
- Early immunosuppressive agents such as cyclophosphamide should be associated to glucocorticoids

All patients

- Consider low-dose aspirin following the recommendations for preventing complications of atherosclerosis

ischaemic events, but it is conceivable that effective suppression of inflammation could also curb ischaemic complications.

In the extremely rare event of the occurrence of intracranial vasculitis secondary to GCA, the prognosis is poor, with a mortality rate of 53% and a median time-to-death of 12 days[86]. Glucocorticoids alone are unable to prevent neurological complications; aggressive treatment with more potent immunosuppressive agents such as cyclophosphamide might be more effective in this case[35,86].

Conclusions

Visual loss and CVAs are the most feared manifestations of GCA and occur in ≤20% and 1.5–7.5% of patients, respectively, usually before glucocorticoid therapy is started. Cranial ischaemic manifestations are early events in GCA and can be prevented by starting glucocorticoid therapy. A previous ischaemic event is the strongest predictor for a subsequent event, although other risk factors are also associated with the occurrence of cranial ischaemic events. Adequate glucocorticoid dosage mostly prevents further cranial ischaemic events; however, although both methylprednisolone pulse therapy and oral glucocorticoids are equally effective in preventing visual complications, neither treatment is effective in improving established visual loss. Fast-track clinics for the diagnosis of GCA might substantially reduce the occurrence of permanent sight loss by reducing delayed diagnosis. Newer agents that are able to effectively suppress inflammation in GCA might also decrease the risk of visual loss and glucocorticoids exposure. A better understanding of the molecular mechanisms involved in the pathogenesis of severe cranial ischaemic complications in GCA should facilitate the development of drugs that are able to selectively inhibit single molecules or pathways involved in these events. Perhaps in the near future these agents could replace glucocorticoids in the treatment of GCA.

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All authors researched the data for the article, provided substantial contributions to discussions of its content, wrote the article and undertook review and/or editing of the manuscript before submission.

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Review criteria
A search for original articles published between 1963 and 2017 and focusing on cranial ischaemic events in giant cell arteritis was performed in MEDLINE and PubMed. Additional papers of interest were retrieved by hand search. The search terms used were 'giant cell arteritis', 'blindness', 'amaurosis fugax' and 'stroke', alone and in combination. All articles identified were English-language, full-text papers. The reference lists of identified articles were also searched for further relevant papers.

Sexual and reproductive health in rheumatic disease

Monika Østensen[1,2]

Abstract | Family size is reduced among patients with rheumatic diseases. The causes for the low number of children are multifactorial and include impaired sexual function, decreased gonadal function, pregnancy loss, therapy and personal choices. Sexuality contributes to quality of life in patients with rheumatic disease, but is often ignored by health professionals. Both disease-related factors and psychological responses to chronic disease can impair sexual functioning. Toxic effects of anti-inflammatory and immunosuppressive drugs can induce transient or permanent gonadal failure in women and men. Furthermore, permanent infertility can be a consequence of treatment with cyclophosphamide, whereas transient infertility can be caused by NSAIDs in women and sulfasalazine in men. These adverse effects must be communicated to the patients, and measures to preserve fertility should be initiated before the start of gonadotoxic therapy. Management of patients of both genders should include regular family planning, effective treatment of high disease activity, sexual counselling, and, if necessary, infertility treatment.

Disturbances of reproductive health cannot just be ascribed to the physical consequences of chronic disease, but require a holistic approach that also includes psychological and cultural factors[1]. Interestingly, a gender-related imbalance exists in studies of reproductive failure in patients with rheumatic diseases: although sexual health aspects are studied with equal frequency in both genders, fertility and gonadal function are more frequently studied in women, and effects of drugs on fertility have been insufficiently studied in both women and men. In this Review, sexual function, fertility and drug effects are discussed in the context of rheumatic disease.

Sexual health

Rheumatic diseases influence all aspects of life, including the quality of sexual life, through their physical and psychological symptoms. The impact of chronic disease is often multifactorial and includes physical components, hormonal imbalance, effects of therapy and psychological alterations[1]. As a consequence, sexual problems among patients with chronic diseases are common and often increase with disease duration[2]. Assessment of sexual function by validated instruments includes measures of frequency of intercourse and sexual desire, arousal, orgasm and sexual satisfaction. Vaginal lubrication in women, and erectile function and ejaculation (retrograde ejaculation and anejaculation) in men are part of the evaluation.

Sexual problems among patients with rheumatoid arthritis (RA) are common[3–6]. Lack of mobility and musculoskeletal pain can restrict intercourse and limit sexual activity. In a study involving 830 patients with RA of both genders, one-third of patients reported that their health status considerably influenced their sexual activity[3]. High levels of fatigue, mental distress, functional limitations, low levels of self-efficacy and male gender were independently associated with perceived problems with sexual activity[3]. In a group of 231 patients with RA, approximately 54% of men and 46% of women reported some kind of sexual dysfunction[4]. Erectile dysfunction in men was related to disease activity, pain and fatigue.

Factors contributing to sexual dysfunction have been investigated in several studies comparing women with rheumatic disease to age-matched healthy women. Disease severity, pain and depression seem to be the most important predictors of sexual dysfunction, affecting desire, arousal, orgasm and satisfaction[5,6]. Impaired sexual functioning was also found in a study of patients with juvenile idiopathic arthritis (JIA), particularly in those with a poor body image[7].

Compared with patients with systemic lupus erythematosus (SLE) and high disease activity who had impaired sexual function, no significant difference in sexual activity was found between women with SLE and no or low disease activity and healthy controls[8]. Other studies showed that women with SLE had a much less enjoyable sex life than healthy women[9–11]. However, the reduction

[1]*Norwegian National Advisory Unit on Pregnancy and Rheumatic Diseases, Department of Rheumatology, St. Olavs Hospital, Trondheim University Hospital, Olav Kyrres Gate 13, N-7006 Trondheim, Norway*
[2]*Department of Rheumatology, Sørlandet Sykehus, Egsveien 100, 4615 Kristiansand, Norway.*
monika.ostensen@gmail.com

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Key points

- The cause of reduced fertility in women and men with rheumatic disease is multifactorial and is related to disease activity, therapy, impaired sexual function and personal choices
- At present, evidence for impairment of fertility is only robust for cyclophosphamide in both genders and for sulfasalazine in men; measurements for preservation of fertility should be initiated in both genders before or during early treatment with cyclophosphamide
- NSAIDs can delay or temporary inhibit ovulation; the choice of type of NSAID, dosage and timing of NSAID administration can reduce the effect on ovulation.
- The first step to avoid unintended infertility is to monitor sexual function and family planning routinely in all patients of fertile age to detect problems related to reproductive health.
- Improvement of reproductive health is achieved by effective treatment of rheumatic disease, comprehensive counselling, and if needed, referral to specialists in sexual medicine and infertility.

of sexual activity was not obviously related to worse genital health nor to disease-specific factors, disease severity or treatment, but rather to psychopathological features and the way patients coped with their disease[10]. In a questionnaire-based study, women with SLE reported a higher rate of sexual dysfunction and impaired partner relationship than healthy women[11]. Poor body image and depression, as well as disturbed social relationships, were associated with sexual dysfunction.

The rate of gonadal and sexual dysfunction is increased in men with SLE (REF. 12). Reduced libido, erectile dysfunction, premature ejaculation and/or anorgasmy and dissatisfaction with sexual life were reported in 20% of young men with SLE and in none of 25 healthy men. In addition, the percentage of pregnant partners was significantly lower in patients with SLE compared with healthy individuals[12].

In a study involving 612 patients with ankylosing spondylitis (AS; 72% of which were men) with a mean age of 51 years, 38% of patients reported that their sexual relationships were affected by their disease in a moderate to extreme way[13]. Poor physical function, pain, higher disease activity, anxiety and depression, unemployment and poor self-efficacy were independently associated with a greater impact on patients' sexual relationships.

Sexual health is rarely addressed by health professionals and is rarely spontaneously reported by patients[14]. Health professionals might feel embarrassed to intrude into the intimate sphere of a patient, and patients might feel ashamed of revealing their sexual dysfunction. However, sexual dysfunction is also common in healthy individuals, affecting 20–50% of the general population and having increasing prevalence with age[15]. Sexual dysfunction can create frustration and distress, and if chronic, lead to anxiety and depression, damaging interpersonal relationships. Unfortunately, sexual problems are often not communicated among couples, and there is a reluctance to assume a sick role, which include denial of sexual needs and limited expression of sexual preferences because of disease symptoms[2]. Sexual problems might result in avoidance of intercourse, which can challenge the stability of a relationship. Although physical symptoms associated with rheumatic disease contribute to impaired sexuality, the psychological consequences of chronic illness often seem to be of greater importance than pain and physical disability. Depression and anxiety seem to be the main factors affecting sexual enjoyment and satisfaction[5,9,13].

Fertility in patients with rheumatic disease

Fertility is defined in women as the ability to conceive within one year of unprotected intercourse[1]. The number of children a woman has depends on the outcome of pregnancy and might be less than expected because of early and late pregnancy losses or neonatal and perinatal death[16]. In men, fertility is related to the number of their children.

Fertility in women with rheumatic disease. Reduced fertility in women with rheumatic disease has been reported in several studies published between 2011 and 2016 (REFS 16–21) (FIG. 1). A question arising from these data is whether reduced fertility is a result of the disease process itself or is related instead to therapy, medical advice or individual decisions on family size.

Fertility was assessed in women with RA and SLE[16]. A total of 55% of women with RA and 64% of women with SLE with a disease onset preceding the completion of family had fewer children than they had originally planned to have. The rate of infertility was higher among women diagnosed with RA during childbearing years than in women who had already completed their families. In addition, disease-related concerns such as deleterious effects of drugs on offspring, ability to care for small children or fear to transmit the disease to children, contribute to limiting family size both in RA and SLE. In women with SLE, the reduced number of children seems related to an increased rate of pregnancy loss rather than to infertility[16].

In a population-based study in Norway, fertility rates in women with RA, JIA or other chronic inflammatory arthritides were compared to a large number of age-matched women from the general population[17]. The proportion of nulliparous women were higher in patients with JIA and other chronic inflammatory arthritides than in healthy women (57.3% versus 40.9% and 30.7% versus 24.5%, respectively). Relative fertility rates were reduced in all patient groups after diagnosis, but not before diagnosis, whereas relative fertility rates adjusted for birth order in women with RA, JIA or other chronic inflammatory arthritides after diagnosis were similar (0.88, 0.84 and 0.84, respectively). Of note, compared with healthy women, fertility was not reduced in female patients with disease onset after the age of 30 years, and an increased interval between subsequent pregnancies was only observed in cases of disease onset after the birth of the first child. Parity was investigated in patients with rheumatic disease in a follow-up study from the same registry[18]. Lower parity was found in 156 patients with RA, 107 patients with other chronic inflammatory arthritides and 75 patients with JIA who were childless at the time of diagnosis compared with healthy women. Most of these patients had high disease

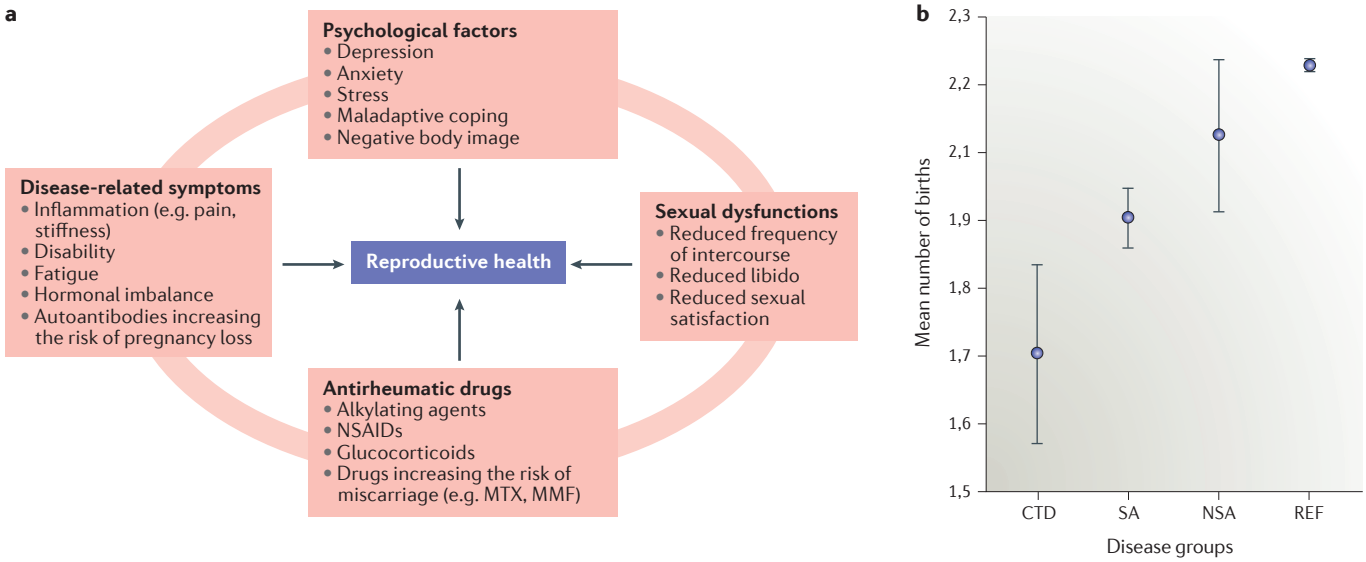

Figure 1 | **Reproductive health in patients with rheumatic diseases.** **a** | Overview of the different factors that influence reproductive health in patients with rheumatic disease. **b** | The graph shows the number of single births in the Norwegian Medical Birth Registry during the period 1967–1995 in different groups of patients. Women with rheumatic diseases were identified according to the International Classification of Diseases (ICD8), whereas all other women formed the reference group (REF). The total number of infants in mothers with rheumatic disease was 3,325, whereas the number of infants in the reference group was 1,396,180. The rheumatic diseases were grouped into three main categories: connective tissue disease (CTD), which comprised systemic lupus erythematosus, Sjögren syndrome, systemic sclerosis and polymyositis or dermatomyositis; specified inflammatory arthritis (SA), including rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis and juvenile idiopathic arthritis; and non-specified inflammatory arthritis (NSA). Except for women with NSA, patients with CTD and SA had a significantly lower number of children than the reference group. MTX, methotrexate; MMF, mycophenolate mofetil. This figure was adapted with permission obtained from Skomsvoll, J. F. *et al.* Number of births, interpregnancy interval, and subsequent pregnancy rate after a diagnosis of inflammatory rheumatic disease in Norwegian women. *J. Rheumatol.* **28**, 2310–2314 (2001).

activity and reduced physical functioning, and all were being treated with DMARDs including biologic agents, indicating an association with more severe disease.

Subfertility with a prolonged time to pregnancy (TTP) has been observed in several studies[19,20]. In a Danish nationwide study involving a cohort of pregnant women, patients with RA had longer TTP compared to healthy women and also received more frequent treatments for infertility[19]. In a Dutch nationwide prospective cohort study on pregnancy, 64 out of 245 patients with RA (31%) who had conceived had a TTP >12 months[20]. These 64 women, together with 40 women who did not become pregnant at all during the study period, were more frequently ACPA-positive and more often used NSAIDs and prednisone. The 40 infertile women had a significantly higher disease activity than those who became pregnant, and they were more often positive for rheumatoid factor. These findings showed that high disease activity, use of NSAIDs and prednisone in daily doses >7.5 mg were associated with prolonged TTP. A follow-up study of the same RA population confirmed that prolonged TTP was associated with the use of NSAIDs, but not with prednisone therapy, although 48% of patients were shown to have unexplained subfertility[21].

Female fertility decreases with age, with a marked decrease of the number of ovarian follicles after the age of 30 years[22]. Several studies have investigated whether chronic inflammation can lead to a decline in ovarian function and premature ovarian failure**.** Impairment of ovarian function could result from different conditions, including autoantibodies to gonadal tissue (autoimmune oophoritis), or from treatment with cytotoxic drugs. Given that regularity of menses is not a reliable marker, anti-Müllerian hormone (AMH), which is secreted by ovarian granulosa cells, has become the preferred marker of ovarian reserve. The serum levels of AMH are fairly stable throughout the menstrual cycle and correlate with the residual follicular pool in women of reproductive age[23].

Conflicting results have been reported regarding the serum levels of AMH in women with RA. AMH serum levels were found to be significantly reduced in patients with rheumatic disease (such as RA, Behçet disease and spondyloarthritis (SpA)) who had not received cytotoxic treatment compared with age-matched, healthy individuals[24]. By contrast, in another study the levels of serum AMH in 72 women with newly diagnosed RA who had not received pharmacotherapy were not significantly different from age-matched healthy women, either at diagnosis or six months after[25]. Premature ovarian failure has been frequently reported in women with SLE, particularly in patients who had been treated with cyclophosphamide (CYC)[26]. However, in the absence of CYC treatment, the prevalence of premature ovarian failure in patients with SLE is consistent with that reported in the general population[26,27]. Analyses of the serum levels of AMH in patients with SLE aged <40 years who had not been

exposed to CYC have shown mixed results. Two studies reported reduced serum levels of AMH in patients with SLE compared with healthy individuals[28,29], whereas other studies have shown no significant difference or correlation between AMH serum levels and disease activity[30,31].

Fertility in men with rheumatic disease. Studies of fertility in men with different rheumatic diseases have involved the evaluation of different parameters, such as gonadal hormonal function, semen quality, testicular alterations and anti-sperm antibodies[32] (TABLE 1). Testicular function can be assessed by measurement of inhibin β A chain (inhibin β), which is produced by Sertoli cells and suppresses the secretion of follicle-stimulating hormone (FSH)[33]. If gonadal function declines, inhibin β decreases and the FSH concentration in serum rises. In men with SLE and dermatomyositis, multiple sperm abnormalities have been detected and a reduction in testicular volume observed , indicating impaired testicular function[34,35]. In 40% of patients with SLE and 20% of men with dermatomyositis, treatment with cyclophosphamide contributed to reduced gonadal function[34,35]. Some patients had anti-sperm antibodies that might reduce sperm motility and function[36]. In addition, the levels of pituitary gonadotropins FSH and luteinizing hormone are higher in patients with SLE than in healthy individuals[34].

Hypogonadism with low levels of bioavailable testosterone has been found in men with RA[37]. Furthermore, chronic inflammation has been shown to affect the hypothalamic–pituitary–gonadal axis[37], and successful treatment with DMARDs to improve testicular function[38]. Normal gonadal function was found in men with AS, with the exception of patients with varicocele, who showed multiple sperm abnormalities[39–41].

Effects of antirheumatic drugs on fertility

Antirheumatic drugs might impair fertility by disturbing ovulation and spermatogenesis or by interfering with the secretion of pituitary and gonadal hormones. In women with rheumatic diseases, subfertility might result from drugs that increase the risk of miscarriages. For many medications that have been used for decades in rheumatology, no data on their effect on fertility are available. Safety studies based on animal data that describe the effect of antirheumatic drugs on fertility are not discussed in this Review because susceptibility of the gonadal system to toxic effects differs among species. In addition, animal studies often involve drug doses that are higher than those used in humans. Human studies investigating the effect of several antirheumatic drugs on female and male fertility are summarized in TABLE 2 and discussed below.

Table 1 | **Summary of studies investigating fertility in men with AS, RA and SLE**

Disease	No. of patients/ no. of healthy individuals	Levels of testosterone in serum	Sperm quality	Levels of inhibin β	Levels of FSH and/or LH	Refs
RA	104/99	Significantly lower in patients with RA than in healthy individuals (9.4 nmol/l versus 11.7 nmol/l, respectively)	NA	NA	Lower levels of LH in patients with RA than in healthy individuals (4.0 U/l versus 5.1 U/l, respectively)	37
RA	41/131	Lower baseline levels in patients with RA than in healthy individuals (16.2 nmol/l versus 23.3 nmol/l, respectively)	NA	NA	Lower levels of LH in patients with RA aged >50 years compared with healthy individuals (4.3 U/l versus 6.2 U/l, respectively)	38
AS	20/24	No difference between patients with AS and healthy individuals	No difference between patients with AS and healthy individuals, except worse sperm quality in eight patients with varicocele	Low level in one patient with AS	No difference between patients with AS and healthy individuals	39,40
AS	21/25	NA	Abnormal in patients with varicocele compared with healthy individuals	NA	NA	41
SLE	34/NA	Low in 15% of patients with SLE when compared with reference values	Abnormal spermatogram in 70% of patients with SLE compared with normal reference values	Low levels in 23% of patients with SLE (11.0 pg/ml)	Elevated levels of FSH in 23% of all patients with SLE	33
SLE	25/25	No significant difference between patients with SLE and healthy individuals	Abnormal spermatograms in 60% of patients with SLE	NA	Levels of FSH and LH in 36% of patients with SLE were higher than those in healthy individuals	12

AS, ankylosing spondylitis; FSH, follicle-stimulating hormone; LH, luteinizing hormone; NA, not available; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

Methotrexate. Methotrexate (MTX) is used for the treatment of musculoskeletal, dermatological, gastrointestinal, oncological and obstetrical conditions at doses varying from 5–25 mg to ≥1 g weekly. When evaluating possible effects of MTX on fertility, the dosage and mode of administration must be considered.

Given its abortifacient properties, MTX is used by obstetricians for termination of ectopic pregnancy. A study involving women treated with a single or up to three doses (50 mg/m^2) of MTX for ectopic pregnancy did not show a significant effect on ovarian reserve[42]. Similarly, no reduction in the serum levels of AMH was observed in women with RA treated with MTX[25]. A prospective, controlled study showed that administration of MTX in the first trimester of pregnancy significantly increases the rate of miscarriages[43]; the miscarriage rate in 188 pregnancies exposed to MTX in the first trimester was 42.5% compared with 14.4% in 136 pregnancies exposed to MTX within 10 weeks before conception[43].

Data on a possible effect of MTX on male fertility are conflicting. Oligospermia and azoospermia have been reported in men treated with MTX as part of a cytotoxic combination therapy for cancer[44]. Case reports have shown either oligospermia and azoospermia or normal sperm quality in men with psoriasis receiving MTX[45]. In a study involving 26 men with psoriasis treated with 25 mg of MTX weekly, no adverse effects on fertility were observed, as assessed by semen analysis, testicular histology and spermatogenic activity[46]. Similarly, a study investigating the effect of TNF inhibitors on spermatogenesis showed that weekly MTX treatment at a low dose did not affect fertility in men with spondyloarthritis receiving combination therapy[47].

Table 2 | **Effect of antirheumatic drugs on female and male fertility**

Drug	Fertility in women	Comments	Fertility in men	Comments
NSAIDs	Non-selective and selective cyclooxygenase inhibitors induce luteinised unruptured follicle syndrome in a dose-dependent and menstrual cycle-dependent manner[55]	The risk for ovulation inhibition can be minimized by discontinuing NSAIDs between days 8–20 of the menstrual cycle[55]	Dose-dependent decrease in quantity and quality of sperm in men taking aspirin or other NSAIDs; reversibility was not studied[56]	No robust evidence indicates that NSAIDs impair spermatogenesis; no studies have been performed in patients with rheumatic disease
Azathioprine	No impairment of fertility[73]	No restrictions on use	No quantitative or qualitative abnormalities of sperm observed in men with IBD[73]	No impairment of male fertility
Sulfasalazine	No impairment of fertility[62]	No restrictions on use	Quantitative and qualitative abnormalities of sperm observed in 40–86% of treated men with IBD[63–65]	Transient infertility and recovery of spermatogenesis 2–3 months after discontinuation of the drug
Glucocorticoids	Disturbance of pituitary–gonadal axis, with effects on the secretion of follicle-stimulating hormone and luteinizing hormone[58]. Prolonged time to pregnancy at daily doses of prednisone >7.5 mg (REF. 20)	No robust evidence indicating that glucocorticoids reduce female fertility	Decrease in serum testosterone levels[60]; no sperm alterations found in men with IBD[73]	Long-term and high-dose prednisone therapy can disturb the pituary–gonadal axis; data regarding impairment of spermatogenesis is lacking
Methotrexate (5–25 mg weekly (low dose))	The rate of spontaneous abortion increases when taken in the first trimester of pregnancy[43]; no effect on ovarian reserve[25]	Must be discontinued before conception[43]	Reversible oligospermia or azoospermia with 7.5–25 mg methotrexate weekly in men with psoriasis or malignancies were reported in a controversial case[45], but no impaired sperm quality in semen and testicular biopsy was reported in a case series of 26 men with psoriasis[46]	No proven adverse effects on spermatogenesis; cryopreservation of sperm before start of therapy is not indicated
Cyclophosphamide	Cumulative dose and age-related gonadotoxic effects; premature ovarian failure at a cumulative dose >10 g[49]	Treatment with a gonadotropin-releasing hormone agonist should be initiated before or early on in therapy	Oligospermia and azoospermia at cumulative doses of 6–10 g (REFS 51,52)	Irreversible testicular damage at high doses. Cryopreservation of sperm should be initiated before start of therapyl; time for recovery of spermatogenesis varies at doses >6 g
TNF inhibitors	No impairment of fertility[74]	No restrictions on use	No quantitative or qualitative abnormalities of sperm observed in men treated short-term or long-term with infliximab, adalimumab or etanercept[47,67,68]	No impairment of male fertility

IBD, inflammatory bowel disease; TNF, tumor necrosis factor.

Cyclophosphamide. In both genders, the toxic effects of CYC on the gonadal system is related to the cumulative dose of this drug, whereas in women, it is also related to age. The European recommendations for the treatment of lupus nephritis suggest low total doses of CYC for induction therapy (preferentially 3–5 g) in order to preserve fertility[48]. In women treated for lupus nephritis, sustained amenorrhoea after a total dose of 3.5–7 g of CYC is rare under the age of 25 years, but increases to 12% in patients aged 26 –30 years and to 25% in patients aged ≥31 years[49]. At a cumulative CYC dose >10 g, women with SLE aged >30 years are at risk of developing premature ovarian failure[26,28].

The levels of serum AMH in women with SLE receiving CYC have been shown to be significantly lower than in patients who did not receive this drug[28,50]. However, another study showed no correlation between the serum levels of AMH and the occurrence of pregnancy[28].

In men with SLE receiving CYC therapy, gonadal damage is reflected by oligospermia and azoospermia, as well as low levels of testosterone and inhibin β and elevated levels of FSH[33]. CYC treatment at cumulative doses >7.5 g/m^2 results in a high risk of permanent infertility[51,52]. In survivors of childhood cancer who had been treated with CYC, recovery of spermatogenesis was sometimes observed many years after treatment[53].

NSAIDs. NSAIDs are either non-selective or selective inhibitors of cyclooxygenase 1 and 2 (also known as prostaglandin G/H synthase 1 and 2), enzymes necessary for prostaglandin synthesis. Inhibition of prostaglandins in the pre-ovulatory phase prevents the rupture of the follicle wall and the release of the oocyte in a phenomenon called luteinized unruptured follicle (LUF) syndrome[54]. Studies involving healthy women and women with inflammatory arthropathies treated with full doses of indomethacin, naproxen, diclofenac, piroxicam, meloxicam or rofecoxib in the pre-ovulatory phase resulted in a prevalence of LUF of 50–100%[55]. In a study involving 14 patients with inflammatory rheumatic disease, 29 women with non-inflammatory musculoskeletal conditions and 449 women not exposed to NSAIDs, LUF syndrome was detected by intravaginal ultrasound in 35.6% of patients receiving NSAIDs compared with 3.4% of women not receiving NSAIDs[55]. Women taking NSAIDs for active inflammatory arthritis developed LUF syndrome significantly less frequently than women taking NSAIDs for inactive rheumatic conditions (15% versus 46.2%, respectively)[55]. Of note, the frequency of LUF syndrome was significantly higher in women receiving the selective cyclooxygenase 2 inhibitor etoricoxib than in women receiving non-selective COX inhibitors such as diclofenac, ibuprofen or ketoprofen[55].

Limited data are available on the effect of NSAIDs (such as aspirin and indomethacin) on male gonadal function. In a study involving 1,376 men without urogenital or other diseases who attended an infertility clinic and were using non-prescription NSAIDs for at least 6 months, dose-dependent decreases in seminal volume, sperm concentration, quality and motility were observed[56]. Another study investigated the effect of pharmacological doses of paracetamol, aspirin and indomethacin on adult human testis[57]. Although the three agents, which are known as 'endocrine disruptors', induced both anti-androgenic and anti-prostaglandinic effects in testicular cells cultured for up to 48 hours, they had very little or no effects on inhibin β production in the testis. Whether NSAIDs can impair fertility in men with rheumatic disease has not been fully investigated. In a study of 20 men with AS treated with NSAIDs, sperm analysis was found to be completely normal[39].

Glucocorticoids. The effect of glucocorticoids on fertility might involve the hypothalamic–pituitary–gonadal axis in both genders[58]. Glucocorticoids can also act directly on the gonads by interacting with the gluco corticoid receptors present on both testicular and ovarian cells[58]. Prolonged treatment with high doses of glucocorticoids can inhibit the release of luteinizing hormone and FSH, which are needed for ovulation and disturb menstruation. A study involving healthy women showed that treatment with triamcinolone suppressed the luteinizing hormone surge at midcycle and inhibited ovulation[59]. Women with RA wishing to conceive showed prolonged TTP in patients treated with >7.5 mg of prednisone daily[20]. By contrast, no such association has been found in women with SLE, although disturbances of the menstrual cycle have been observed in patients exposed to high dose of glucocorticoids[48].

In men, high doses of glucocorticoids can reduce the levels of testosterone and might also decrease sperm concentration[58,60,61]. However, the effect of administration of low (<10 mg daily) or high levels (>10 mg daily) of prednisone over a prolonged period of time on testicular function in men with rheumatic disease has not been fully elucidated.

Sulfasalazine

Sulfasalazine has no adverse effects on female fertility and is not teratogenic; therefore, this drug can be administered during pregnancy[62]. In men, sulfasalazine can induce transient infertility with oligospermia, abnormal morphology of sperm cells and reduced sperm motility. Plasma levels of steroids and gonadotropins remain normal during sulfasalazine therapy[63]. In men with inflammatory bowel disease being treated with sulfasalazine, 40–86% had abnormal sperm function, with reduced motility being the most frequent abnormality observed[63,64]. Recovery time for normal sperm quality varies between 1 and 3 months after discontinuation of sulfasalazine therapy. The sulfapyridine moiety of sulfasalazine is thought to be responsible for the effect on spermatogenesis given that replacing sulfasalazine with mesalazine in patients with IBD resulted in improved sperm quality[64].

TNF inhibitors. In female patients, TNF inhibitor therapy has not been associated with a reduced number of pregnancies or children in studies with adequately sized control groups[62]. Given the physiological role of TNF in spermatogenesis, some concern exists for treating male patients. TNF is produced by testicular germ cells, and at physiological doses TNF inhibits apoptosis and

promotes survival in these cells[65]; however, high doses of TNF impair spermatogenesis[66]. Studies comparing men treated with TNF inhibitors with disease-matched and/or healthy individuals did not show impairment of sperm quality either following short-term or long-term treatment[47,67,68]. Two studies actually showed that the sperm quality in patients with SpA receiving long-term anti-TNF therapy was significantly better than that of untreated disease-matched controls[47,67].

Counseling and managing

Fertility and sexual function are often neglected areas in the care and management of patients with rheumatic disease[69]. Several barriers can prevent open communication on reproduction issues, including insufficient skills, lack of training and lack of access to specialized services. One study showed that doctors and other health professionals might not feel comfortable to discuss sexuality with their patients because they have not been trained in these issues[70]. In addition, counselling is thought to be time-consuming and not easy to fit into the limited time frame of a consultation. This problem can be solved by assigning these tasks to specially trained nurses, occupational therapists or psychologists of the interdisciplinary team. Some patient associations such as the Arthritis Foundation in the US and Arthritis Care in the United Kingdom offer brochures to deal with sexual problems in patients with rheumatic disease[71]. Referral to specialists in urology, gynecology or sexual medicine might also help in the improvement of a patient's sexual life. A major goal for restoring sexual and reproductive health in patients of both genders is the achievement of optimal disease control. In this regard, the introduction of new treatment strategies such as 'treat-to-target' approaches and the use of combination therapies might have major advantages.

When fertility problems are reported, referral to a reproduction specialist should be considered. The patient should be informed that pregnancy might still be achievable even when markers of gonadal function indicate impaired reproductive function such as reduced ovarian reserve or impaired spermatogenesis. In principle, only one healthy sperm cell and one healthy egg are sufficient for procreation, and the chance to have children is greatly increased by assisted reproduction techniques.

Drug therapy can influence fertility either because of teratogenicity or gonadal toxicity. In the first case, pregnancy must be postponed and a safety interval between drug discontinuation and conception kept. In the second case, impairment of gonadal function must be weighed against any deleterious effect of active, untreated disease on sexuality and fertility, and must be discussed with the patient. In some cases, the type, dose and timing of drug treatment can reduce adverse effects on fertility. For example, treatment with a non-selective cyclooxygenase inhibitor at the lowest effective dose and with a drug-free interval around the mid-cycle minimizes the risk of inhibiting ovulation[55].

For a cytotoxic drug like CYC, the possibility of preserving fertility must be explained to the patient, and strategies such as concomitant gonadotropin-releasing hormone agonist therapy in women, or cryopreservation of sperm in men, should be initiated. Unfortunately, these options are not always considered[70].

Counselling female patients about the optimal time for conception often includes the advice to postpone pregnancy because of active disease, organ damage or treatment with teratogenic drugs. However, unintended adverse effects might include a prolonged TTP or even infertility, particular in women aged >30 years. Comprehensive information and shared decision making in regard to therapy can help to avoid unwanted childlessness.

Conclusions

The impaired reproductive function observed in patients with rheumatic disease derives from several factors, some related to the physical disease process and some related to coping with a chronic disease. The interaction between general ill health, pain and functional disability with a poor body image, anxiety and depression in a man or woman is a major reason for sexual dysfunction. Despite the negative impact of sexual problems on quality of life, issues of sexuality are often ignored or avoided by health professionals in contact with their patients.

The population-based studies discussed previously indicate that fertility in women with rheumatic disease is reduced compared with healthy women of a similar age. However, the underlying causes are not clear, and prospective, controlled studies are needed. Disease-related factors such as pregnancy loss (including early and late miscarriages), neonatal and perinatal death as well as the presence of autoantibodies that are harmful for pregnancy and to the fetus seem to contribute to reduced fertility in women with rheumatic disease. The reason for a prolonged time to achieve pregnancy is less clear, particularly when no detailed assessment of fertility is performed in the patient and her partner; reduced ovarian reserve as well as drugs that inhibit ovulation or cause damage to the ovaries might play a role. In addition, medical advice recommending a period of remission before conception, therapy with teratogenic drugs, functional impairment and personal concerns might all result in the postponement or avoidance of pregnancy. Patients with rheumatic disease sometimes consider voluntary childlessness regardless of disease-related physical problems owing to concerns about adverse reproductive outcomes or fear of transmitting disease.

Interestingly, aspects of fertility in male patients with rheumatic disease have been a neglected area of research. Studies in male patients have shown impaired gonadal function reflected by reduced sperm quality and sometimes lowered testosterone levels. No population-based studies have investigated whether men with rheumatic disease have a reduced number of children. A male patients' view on family size or on concerns in regard to becoming a father has not been recorded.

Data on the effects of antirheumatic drugs on fertility are limited. The influence of pharmacotherapy on fertility is often difficult to separate from confounding factors that are related to either disease, or age and lifestyle. In rheumatology, the only drug that induces a substantial

reduction of fertility in both genders is CYC. Notably, the only antirheumatic drugs listed as impairing male fertility by the FDA are CYC and sulfasalazine[72]. For other drugs, current studies often have serious shortcomings as they are either inadequately designed to investigate fertility or do not consider possible confounders such as fertility problems of the partner, disease activity and severity, lifestyle and anthropometric factors, previous urogenital disease, medical advice or personal choices. Another issue in these studies is the lack of disease-matched control groups. Adverse effects of drugs are sometimes predicted by theoretical concerns, not proven effects. One should also be aware that drug effects studied in healthy individuals might not reflect drug effects in patients with chronic inflammation. This phenomenon has been shown for NSAIDs , which induce less ovulation inhibition in women with active joint inflammation compared with women being treated for low back pain[55]. A major aspect for future research is the reproductive function in men with rheumatic disease. National medical birth registries could provide data on the number of children in men with rheumatic disease who received or not received treatment with immunosuppressive drugs, and these data could be compared with that on healthy male population. Adequately designed studies that include a non-exposed disease-matched control group and healthy age-matched controls are needed for investigating the effect of drugs on male and female fertility. The lack of knowledge is most pronounced in male patients and particularly concerns 'old drugs' that have beeen used for decades, such as NSAIDs, glucocorticoids and MTX. In addition to researching reproductive function in patients with rheumatic disease, a change of attitude on the side of health professionals is needed, away from being a 'taboo' topic and towards actively addressing sexuality and fertility. Awareness of the importance of these aspects and willingness to address them when meeting a patient with rheumatic disease is a first step to solving these problems.

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OPINION

Antinuclear antibody testing — misunderstood or misbegotten?

David S. Pisetsky

Abstract | Antinuclear antibodies (ANAs) are a diverse group of autoantibodies that recognize nuclear macromolecules and their complexes. ANAs represent key biomarkers in the evaluation of rheumatic diseases, most prominently systemic lupus erythematosus (SLE), and ANA testing is commonly performed in the clinical setting. In addition, ANA testing is now used to assess eligibility for participation in clinical trials of new therapeutic agents for SLE. ANAs can be assayed by various techniques, with the fluorescent ANA assay often viewed as the gold standard. Whereas a positive ANA test represents a classification criterion for SLE, up to 20–30% of the healthy population, depending on the assay used, is positive for an ANA, complicating the use of this test for diagnosis or the detection of preclinical autoimmunity. Furthermore, ANAs might be expressed in SLE less commonly than often thought. This Perspectives article discusses important questions about the use of ANA testing in both the clinical and research settings.

Antinuclear antibodies (ANAs) are a diverse group of autoantibodies whose detection is key to the evaluation of patients with the broad range of rheumatic diseases[1]. ANAs target macromolecular components in the cell nucleus and can bind to DNA, RNA and proteins, as well as complexes of nucleic acids with proteins. The number of different ANA specificities is large and, whereas some antibodies are highly associated with particular diseases, others are expressed more widely among patients[2–4]. The association between ANAs and certain disease entities suggests that these antibodies could be useful biomarkers for screening and diagnosis and could provide insights for understanding disease mechanisms.

Although ANA testing has been central in rheumatology for over 50 years, many aspects of these popular biomarkers remain a matter of uncertainty, contention and even controversy. Furthermore, the clinical relevance of both ANA positivity and negativity has evolved and shifted over time[5]. ANA positivity was initially seen as a notable, indeed intriguing, feature of systemic lupus erythematosus (SLE), distinctive enough to become a criterion for classification of the disease. At present, ANA positivity occurs so commonly in patients with musculoskeletal complaints and vague symptomatology that a positive result might be neither revealing nor informative; indeed, ANA positivity might muddle an otherwise sensible and parsimonious diagnostic work-up[6,7].

This Perspectives article considers salient aspects of ANA testing and addresses how to utilize the sometimes bewildering information provided by this test, focusing on its use in SLE as an example of an ANA-associated rheumatic disease. In this article, the term 'ANA' is used in a general sense, notwithstanding the cytoplasmic location of some of the antigens (such as Jo-1 or histidyl tRNA synthetase) recognized by these antibodies and, furthermore, that immunofluorescence assays (IFAs) using human epithelial type 2 (HEp-2) cells enable the detection of antibodies to cytoplasmic, mitotic and nuclear molecules[8,9]. Thus, although some autoantibodies under the rubric of ANA do not bind to nuclear molecules, this term is used herein because it is embedded in the history of the field and the scientific literature.

The function of target antigens

The molecules bound by ANAs are, as the name indicates, primarily found in the cell nucleus but vary in biochemical composition and nuclear localization; as noted above, antibodies to certain cytoplasmic proteins might also fall into this category. Nuclear proteins recognized by ANAs have essential intracellular functions such as replication and transcription, and thus are structurally conserved among species. Therefore, ANAs, although defined as autoantibodies, are able to bind to nuclear antigens from different species, as they recognize parts of molecules sharing a similar sequence or structure[1].

ANAs bind a very wide range of molecules but can be divided into two groups in the context of SLE and related diseases. The first group consists of antibodies that recognize DNA, histones and nucleosomes. Antibodies to nucleosomal components are closely related, although only anti-DNA antibodies are routinely assayed. The second group includes ANAs that bind to complexes of RNA with RNA-binding proteins (RBPs); however, these antibodies are directed to the protein components of complexes such as the small nuclear ribonucleoproteins (snRNPs). Examples of ANAs recognizing RBPs include anti-Sm, anti-RNP, anti-Ro and anti-La antibodies[1]. Anti-Sm and anti-RNP antibodies, which are commonly expressed in the same patient, bind to protein components of snRNPs or complexes of snRNP with RNA. Anti-Ro and anti-La antibodies are also commonly expressed together, although antibodies recognizing two different anti-Ro antigens exist. Antibodies against Ro60 bind to the protein component of a complex comprising small cytoplasmic RNA molecules; by contrast, the anti-Ro52 antibody recognizes a member of the tripartite motif (TRIM) family, which is a ubiquitin ligase that does not form RNA or protein complexes[10,11]. Other antigens recognized by ANAs, such as ribosomal P proteins and the Ku protein, which can bind DNA strand breaks as a dimer[12,13], do not fit in this categorization, which has been most informative for SLE.

Although the nuclear antigens targeted by ANAs are usually present in the cell nucleus, these molecules are mobile and can

translocate to the cytoplasm as well as to the extracellular space. These translocation events can occur during cell death, most prominently apoptosis. During apoptosis, nuclear molecules can migrate into structures called blebs, which can detach from cells in the form of microparticles. The translocation of nuclear antigens might contribute to their immune properties and ability to induce responses[14].

In the extracellular space, nuclear antigens can form immune complexes with ANAs. These complexes can stimulate immune responses, including the production of type I interferon. The mechanism for this induction probably involves the cellular internalization of the complexes, which provides access of DNA or RNA to internal nucleic acid sensors including Toll-like receptors[15–17]. Thus, nuclear molecules, as immune complexes, can exert important pathogenic activities. Because of the immune activity of these complexes, ANA testing, especially for antibodies of certain specificities (such as anti-DNA and anti-RBP antibodies), can provide valuable information on pathogenic pathways that might be involved in various rheumatic diseases[18,19].

ANA specificity in different diseases

Although ANA testing is useful to assess the likelihood of a diagnosis of rheumatic disease, much more relevant information comes from the identification of the target antigens bound by ANAs, with certain antibodies being strongly associated with particular diseases. These associations are peculiar because the antigens bound by ANAs are present in all cells of the body. However, the manner in which such antibodies lead to distinct clinical patterns is unknown. Important associations include anti-DNA and anti-Sm antibodies with SLE; anti-topoisomerase I antibodies with progressive systemic sclerosis; anti-centromere antibodies with a limited cutaneous form of systemic sclerosis; and anti-Jo-1 antibodies with myositis[1–4,20–24]. The antibodies in myositis are in fact quite diverse but require special testing[24]. In contrast to the disease specificity of some ANAs, antibodies to Ro60, Ro52 and La antigens, despite being an important feature of Sjögren syndrome, also commonly occur in SLE and rheumatoid arthritis (RA). Moreover, anti-Ro52 and anti-Ro60 antibodies also occur in subacute cutaneous SLE and neonatal SLE syndromes[10,11]. Antibodies to RNP antigens, which form snRNP complexes, are commonly co-expressed with anti-Sm in SLE and might actually be found with a higher frequency in this disease[20–22]. Nevertheless, positivity to anti-RNP antibodies is not considered a classification criterion for SLE diagnosis because these antibodies are also found in mixed connective tissue disease (MCTD)[23]. MCTD has features of different rheumatic diseases, with the presence of anti-RNP antibodies considered important in the diagnosis.

Table 1 | **Association between ANAs and clinical symptoms in patients with SLE**

ANA	Clinical symptom(s)
Anti-DNA antibody	Renal disease
Anti-P antibody	Neuropyschiatric manifestations
Anti-Ro antibody	Neonatal lupus syndrome and sub-acute cutaneous lupus
Anti-Ro and anti-La antibodies	Sicca symptoms
Anti-RNP antibody	Raynaud phenomenon

ANA, antinuclear antibody; RNP, ribonucleoprotein; SLE, systemic lupus erythematosus.

In patients who are positive for ANAs, depending on the clinical and laboratory findings, the presence of a specific ANA increases the likelihood of a diagnosis of disease. Similarly, the absence of ANAs with disease specificity diminishes the likelihood of a diagnosis of disease. Testing for the presence of ANAs that are present specifically in healthy individuals would be particularly useful as it would address the issue of false positives and the lack of clinical significance of conventional ANAs. Antibodies to DFS70, which are discussed in the next section, might fulfil these criteria. TABLE 1 summarizes some of the clinical associations of differents ANAs with disease manifestations.

ANA assays

Since the incorporation of ANA testing into routine patient evaluation, the technology for antibody assessment has evolved and encompassed a variety of immunochemical approaches.

Immunofluorescence assay. For about 50 years, the fluorescent ANA assay (also known as indirect IFA) has been the major technique in ANA testing. As an important application of fluorescent antibody technology, the IFA was developed to provide an assay more robust and sensitive than the 'Lupus Erythematosus (LE) cell' assay[25]. In the LE cell assay — now rarely performed — a blood specimen is disrupted, incubated, and then spotted onto a glass slide for staining with Wright's stain. LE cells result from the phagocytosis of the nucleus from another cell that has been opsonized by an ANA and complement. The presence of bound IgG antibodies and complement facilitates the phagocytic uptake by the neutrophil. The key factor of the LE test is the type of ANA involved.

IFA was developed to enhance the sensitivity and reproducibility of ANA testing, and to detect more ANAs than those detected in the LE cell assay. IFA is simple in principle, and involves incubation of serum or plasma samples with a source of cells, either a tissue section or a cell line fixed to a glass slide (FIG. 1). At present, the HEp-2 cell line is used for this assay because it displays a wide variety of antigens. In IFA, the presence of antibodies is assessed using a fluoresceinated anti-IgG reagent, and positivity is assessed by visual inspection[26]. In general, the frequency of ANA positivity in the blood of patients with SLE is considered to be very high (95–99%)[1–5,27]. Depending on the definition of ANA, a substantial frequency of positive results has also been reported in the blood of patients with other rheumatic diseases such as RA, myositis and systemic sclerosis[2].

The patterns of fluorescence observed in IFA might also provide insight into the specificity of ANAs given the characteristic nuclear location of target antigens. In this regard, given that entire cells are used for antibody detection, antibodies to cytoplasmic, mitotic and nuclear molecules can also be detected; this possibility can lead to confusion in terminology as well as uncertainty in the use of ANA positivity as a criterion for disease diagnosis and classification. Common patterns detected by IFA include homogeneous, speckled, rim and nucleolar patterns (FIG. 1). Since a multitude of topological features exist in these patterns, technical skills are needed for their recognition[8,9,28].

ANA positivity is just about essential for the diagnosis of SLE, and IFA is often regarded as the 'gold standard' for serological testing. Unfortunately, the gold standard does not have the brightness and lustre often ascribed to it. The performance

of IFA can indeed be subject to variability related to the assay kit used, conditions of cell fixation, cellular concentration of antigens and the specificity of the anti-IgG reagents. Another issue with IFA is the starting dilution of sera used for testing. Given that even normal sera can result in ANA staining when used at low dilutions, the initial dilution for routine testing is usually 1:40 or 1:80, for example. Still higher dilutions can be used for the initial screening, but the frequency of positive results in samples from patients with the disease will be decreased. Thus, the definition of positive and negative results in IFA testing is operational, and depends on the cutoff used in terms of the starting dilution of the serum[29–32]. In addition to reducing the number of positive results in a control population, the choice of the starting dilution for screening can also influence the number of positive results in patients with disease.

By its nature, IFA is a visual test and depends upon the observer, although computerized approaches based on digital images can also be used for ANA detection[33–35]. Especially for sera with low titres of antibodies, assignment of positivity might differ among different observers, and throughput can be low. Of note, the amounts of certain autoantigens in HEp-2 cells, such as Ro60, can limit the detection of antibodies against this molecule. A modified cell line called HEp2000 can be used to increase detection of anti-Ro antibodies by IFA. In this cell line, the expression of Ro60 is increased because of a transfected Ro60 complementary DNA, and the pattern of expression of Ro60 can differ from that of untransfected cells[36,37]. The low expression of certain autoantigens can lead to a situation in which IFA testing is negative whereas another approach, such as ELISA, is positive[38].

Beyond issues in determining a threshold for positivity, an important limitation of ANA testing by IFA concerns the frequency of positivity in healthy individuals. Indeed, depending on the assay kit, the range of positive tests in individuals without disease can be as high as 20–30%. In some studies, the frequency of positive ANA tests has been shown to be higher in women than in men, and racial and ethnic differences have been shown to influence results[39,40]. Age might not be an important factor given that children can also show significant ANA responses[41]. The reasons why so many healthy individuals are positive for ANAs are currently unknown; on the one hand, ANA positivity could suggest an underlying propensity for autoimmunity, with ANA expression the first step on a path that leads to autoimmunity[42,43]. On the other hand, ANA positivity could include a high frequency of false positives, with antibody binding to fixed (and denatured) nuclear molecules as an accidental crossreactivity.

Although the reason behind positive ANA results in healthy individuals is not fully understood, the expression of antibodies to an antigen called DFS70 might explain at least some of the positive results of ANA testing[44,45]. Anti-DFS70 antibodies were originally recognized to elucidate an IFA binding pattern called dense fine speckles (DFS), which can be confused with the homogeneous pattern. This antigen was termed DFS70 because initial studies indicated that antibodies producing this pattern bound to a 70 kD protein. Subsequent studies demonstrated that the protein recognized by these antibodies is PC4 and SFRS1-interacting protein (also known as lens epithelium-derived growth factor (LEDGF)). Even though antibodies to DFS70 have been associated with a variety of clinical conditions, they commonly occur in healthy individuals. Importantly, low expression of these antibodies might occur in patients with autoimmune disease characterized by ANA production[44,45]. Therefore, the presence of anti-DFS70 antibodies might exclude the diagnosis of an autoimmune disease, although the possible association with other conditions affects the manner in which a positive test is interpreted in the context of patient evaluation.

The high frequency of positive ANA test results in healthy individuals is accompanied by an unexpectedly low frequency of positive ANA test results in patients with SLE, especially those with established disease. Whereas ANA positivity has been considered almost invariable in patients with SLE, experience with clinical trials of new therapeutic agents in the past decade has indicated that ANA negativity might be more common than previously realized. Prominent examples are the clinical trials for the development of belimumab, a monoclonal antibody targeting B cell activating factor (BAFF; also known as TNF ligand superfamily member 13B). The phase II belimumab study did not demonstrate efficacy of this drug, although the patient population had a high frequency of ANA negativity (approximately 20–30%), a result that was very surprising[46]. The explanation for this high frequency of negative ANA tests is not fully known. It is possible that many patients included in the trial did not, in fact, have SLE, despite some compatible signs and symptoms. Another possibility is that these patients had been serologically positive initially but lost ANA production because of prior treatment or the passage of time. Another explanation is laboratory variation associated with the assay kits used or with different observers. For the phase III belimumab studies, the sponsor established as an entry criterion the positivity for either ANAs or anti-DNA antibodies. These studies were successful, suggesting that enrichment for serologically active patients provides a more appropriate population to demonstrate efficacy[47–49]. With this strategy, belimumab received approval as a treatment for active SLE.

Several studies indicate that the bedrock belief that ANA positivity is almost invariable in SLE might not be as solid as usually considered. Studies have demonstrated that ANA negativity occurs not infrequently in patients with SLE who meet classification criteria and are receiving care in centres with expertise in diagnosis and treatment[50,51]. The number of patients with SLE who are negative for ANAs can range from 5% to 20% depending on the assay kit used and the demographic features of the population under study. Given that many manifestations of SLE (such as nephritis and interferon production by plasmacytoid dendritic cells) probably arise from ANAs via their participation in immune complexes, the immune mechanisms driving disease in ANA-negative patients are currently unknown[15–17]. Certainly, some of the cardinal features of SLE (for example, increased interferon production) could arise from genetic defects in signalling pathways that lead to enhanced cytokine production. Chronic viral infection could also lead to high levels of cytokines, which can promote disease manifestations such as arthritis, fatigue and nervous system disturbances.

Although ANA production might cease in some patients with SLE, it is also possible that the finding of seronegativity results from inadequate sensitivity of the assay kits or the inherent difficulty of IFA in detecting certain antibodies (such as anti-Ro60 antibodies). Together, these findings have led to an interest in developing other assays that might detect ANAs more reliably and might provide higher throughput, less demand for experienced personnel and higher cost efficiency.

ELISA. ELISA is one of the most popular and versatile analytical techniques in medicine, and is able to measure a multitude of analytes.

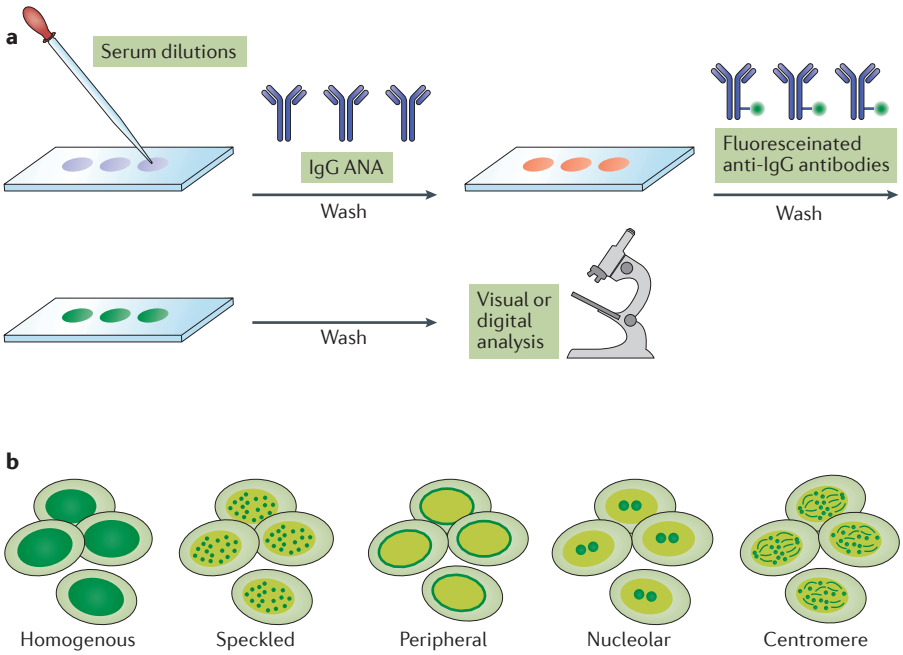

Figure 1 | **IFA testing for identifying the presence of ANAs. a** | In the top panel, the experimental procedure of an indirect immunofluorescence assay (IFA) is illustrated. A slide with tissue culture cells is exposed to dilutions of serum. Following washing steps to remove unbound antibodies, the slide is incubated with a fluoresceinated anti-IgG reagent. Following another washing step, fluorescence microscopy is performed. At present, in most laboratories, a technician visually inspects the slide to determine the presence and pattern of fluorescence. In determining the positivity of a sample, the dilution in which fluorescence is still visible is assessed. This end-point titre provides a quantitative measure of the amount of antinuclear antibodies (ANAs) present. In addition to visual inspection, the presence of fluorescence can be determined from digital images. **b** | The bottom panel illustrates some of the more common staining patterns of ANAs. Each pattern is associated with certain ANAs and, therefore, can occur more commonly in association with certain diseases. The results of IFA can be reported in terms of the end-point titre and staining pattern. For some sera, a mixture of different ANAs is present and, depending on their relative titre, more than one pattern can be observed. For example, a serum can be characterized as 1:640 homogeneous and 1:2,560 speckled. As IFA kits can differ in features related to the cell line, conditions of fixation and properties of anti-IgG reagents, the results can vary. Therefore, it is important to know the characteristics of different assays for interpreting their results. In general, a titre of >1:40 or 1:80 is considered significant meaning that the serum is considered to be ANA positive.

To create an antigenic substrate that can be used to detect the presence of an antibody, a diluted solution of a protein (or another molecule) is incubated with a plastic surface, usually a 96-well plate. The antigen can stick to the surface nonspecifically, becoming tightly bound in a way that allows for multiple washing steps after incubation with an antibody source and an enzyme-conjugated anti-IgG reagent; the enzymes are usually either peroxidase or alkaline phosphatase. The final step of an ELISA involves the addition of a substrate that is converted into a detectable signal by the enzymes.

The development of an ELISA for ANAs is challenging given the large number of different components in a nuclear extract whose concentrations are unknown. Furthermore, within a complex mixture, antigens can compete for binding to the plastic surface, affecting the representation of different nuclear molecules as antigenic substrates. Rather than nuclear extracts, a more defined mixture of purified components can be used, which ensures the presence of relevant antigens of interest. The two approaches can also be combined by 'spiking' a nuclear extract with purified components.

Many ELISAs have been developed and validated for ANA detection[52–57]. The advantages of this approach include ease of use as well as the possibility to perform quantitative and high-throughput analyses. The main disadvantage relates to uncertainty in the content of certain antigens and lack of information on the specificity of antibodies leading to a positive response (which can be useful to distinguish disease entities). Thus, in an ELISA, the binding properties of different antibodies can be very similar despite major differences in their specificity. Although IFA does not provide precise information about the specificity of antibodies in a serum, this technique can at least indicate the identity of antibodies through their binding pattern. Depending on its design, the properties of ANA ELISA will be similar to those of IFA in terms of the frequency of positive test in patients with disease and the frequency of negative results in healthy individuals.

ELISA can be also used to detect specific ANAs (such as anti-Ro and anti-La antibodies), but this information can be readily provided by multiparameter assays such as line immunoassays or bead-based multiplex assays (discussed below). ELISA is still regularly used for the detection of anti-DNA antibodies over the course of SLE because of the frequent correlation of the levels of these antibodies with disease activity. Whereas the ELISA can reliably detect anti-DNA antibodies, the antibody populations measured in this type of assay might differ from those measured in other assay formats. Indeed, ELISA tends to detect antibodies with lower avidity than those detected by the Farr or *Crithidia luciliae* assays, and leads to a higher frequency of positive results in a patient population[58–61]. The importance of these differences is not clear because the relationship between specificity, avidity and pathogenicity of antibodies has not yet been clarified.

Multiplex assays. A number of multiplex assays are available for the simultaneous detection of ANAs recognizing nuclear autoantigens associated with different rheumatic diseases. Among these assays, line immunoassays are a useful technique for detecting the presence of antibodies to specific autoantigens[62,63]. In line immunoassays, a limited selection of antigens (such as purified proteins, recombinant proteins or synthetic peptides) is bound in parallel lines to a nylon membrane in order to provide a substrate for antibody detection. The nylon membrane is then exposed to a dilution of sera from a patient, with the presence of antibodies detected by an anti-human IgG reagent conjugated to an enzyme. This system can be automated and has the advantage of providing the simultaneous detection of several autoantibodies with diagnostic relevance.

Multiplex assays with addressable beads are a more recent and technologically sophisticated approach to detect antibodies against multiple independent nuclear antigens. This assay utilizes a series of

beads of distinct immunofluorescence intensities, each coated with a different purified antigen. The presence of antibodies to different antigens can be determined by flow cytometry in both a quantitative and qualitative fashion by analysing the antibodies bound to each of the different beads. If the test shows positivity for antibodies to any antigen, the serum is considered to be 'screen positive', a preliminary indication of ANA reactivity. The result of a multiplex assay can be confirmed by conventional IFA[64–67].

The utilization of bead-based multiplex assays by clinical laboratories is a matter of considerable debate because this approach is different from that of conventional IFA or even ELISA with nuclear extracts. Similarly to the line immunoassay, the multiplex bead-based assay assesses the binding of only a limited subset of ANAs, and thus can miss many of the less-common specificities or, for example, specific types of autoantibodies from patients with myositis. Such patients can produce a variety of antibodies to transfer RNA (tRNA) synthetases and other proteins, but one of the commonly used multiplex assays allows the detection of antibodies only to Jo-1 or histidyl tRNA synthetase[68]. Similarly, whereas patients with SLE can produce as many as 200 different ANAs, the multiplex assay allows the detection of only a few of the most common antibodies, such as those recognizing DNA, chromatin, Sm or RNP[69].

The aim of line immunoassays and bead-based multiplex assays is to make ANA testing more specific and quantitative, allowing detection of autoantibodies that are specifically associated with common rheumatic diseases. The results of these assays are more immediate and direct than those from IFA testing. As these assays involve the use of defined antigens, some of the ambiguity and uncertainty of IFA is reduced, especially with sera from healthy individuals who produce ANA with undefined specificity. This property could be valuable because one of the first steps in assessing the significance of a positive ANA test is to determine whether an antibody of known specificity is present. However, line immunoassays or multiplex bead-based assays will not be able to detect some pathogenic ANAs; in this regard, if the suspicion of a rheumatic disease is high, a conventional IFA or other types of assays can also be performed. Despite their ease of use, the multiplex assays do not fully eliminate problems related to the detection of autoantibodies in healthy individuals or patients being evaluated for a possible inflammatory or autoimmune disease; ANA positivity should always be interpreted in the context of the clinical situation and the pre-test probability of a disease.

Other technologies such as chip-based assays can provide serological assessments in greater detail. These assays enable measurement of antibodies to literally hundreds of antigens, which can include conventional targets of ANA testing as well as other proteins of immunological relevance[70,71]. The broad analysis of autoantibody binding provided by chip-based assays provides a more complete and precise picture of autoreactivity than was previously possible. These assays are still evolving because the nature of the antigens in these assays (such as peptides or recombinant proteins) might influence the spectrum of the antibodies detected, especially for those antibodies directed to conformational determinants. Conformational determinants are higher-order structures that require the presence of a protein, or result from the interaction of proteins with other proteins or molecules that might occur in complexes. It is possible that further refinements of these assays and the inclusion of additional antigens to the array will enable clinically important distinctions such as pre-autoimmunity (discussed in the next section), as disease-associated ANAs frequently bind to diverse antigens, which an array can represent.

The conundrums of ANA specificity

Any approach to ANA testing must take into account three well-established facts; firstly, the frequency of ANA positivity determined by IFA testing in an otherwise healthy population can be high; secondly, rheumatic diseases are very uncommon in the population; thirdly, most people who are ANA-positive will never develop a rheumatic disease. These facts alone suggest caution in the use of ANA testing as a screening tool in evaluating patients for inflammatory diseases, as the vast majority of ANA results will be false positives. Even with the ELISA or multiplex assay, the occurrence of false-positive results can become considerable because these tests are performed very often in patient evaluation, even in settings where the pre-test probability of an autoimmune condition is very low. In medicine, a high frequency of false positives in a test can lead to excessive, and often unnecessary, testing, costs and patient concerns. For example, many rheumatologists have encountered patients who have been told that they might have SLE because of a positive ANA test, which causes persistent anxiety and overtreatment. Once a diagnosis has been communicated to a patient, it is often difficult to convince them that the physician who made the diagnosis was mistaken.

Whereas the more selective use of ANA testing would be a considerable advance in the clinical practice, screening of patients could be also valuable for the early detection of a rheumatic disease, especially SLE. As is now recognized, patients who develop SLE can have serological positivity for ANAs years before a clinical diagnosis, a state known as 'pre-autoimmunity' (REFS 72,73). In the pre-autoimmunity phase, abnormalities in the immune system allow the emergence of autoreactivity, which manifests as ANA production, but other factors that lead to clinical disease manifestations have not yet occurred[74,75]. The use of immunomodulatory therapy at this stage could prevent the development of clinical disease and the inflammation and damage that occur as a consequence.

The identification of individuals with pre-autoimmunity could be performed in a targeted way by looking at first-degree relatives who are at increased risk of disease, probably owing to shared genes or environmental factors[76]. This strategy might be useful in clinical studies, but, in the real world, it is unlikely to happen. Rather, the possibility of identifying pre-autoimmunity will probably depend on the use of ANA screening in situations where the pre-test probability for a true positive result seems low (for example, nonspecific complaints of fatigue or arthralgia). In such situations, there are at least two ways to determine whether the test is worth pursuing: the titre and binding pattern of ANAs through IFA[39,77], and the identification of specific ANAs such as anti-DNA, anti-Ro60 or anti-La antibodies. Given that most healthy people positive for ANAs do not express ANAs associated with a disease, the use of a multiplex or chip-based array assay can provide useful information about whether an individual should be observed more carefully. A positive test for disease-associated ANAs can also lead to further evaluation or interpretation of symptoms.

ANAs versus anti-DNA antibodies

In the development of belimumab, the entry criteria for participation in the phase III clinical trials included positivity for either ANAs or anti-DNA antibodies, suggesting that these antibodies provide

similar biomarker information (such as disease activity). In fact, the diagnostic and prognostic significance of these autoantibodies varies in different individuals; whereas ANA positivity is high in the general population, antibodies to double-stranded DNA are highly specific for the diagnosis of SLE[78,79]. This specificity of anti-DNA antibodies makes these autoantibodies useful for patient classification in the study of disease mechanisms, as well as for the diagnosis of patients in the clinical setting. Furthermore, in patients with SLE, levels of anti-DNA antibodies can fluctuate over time together with disease intensity, suggesting their direct role in pathogenesis, especially in nephritis[80]. On the one hand, this fluctuation makes anti-DNA antibodies useful as biomarkers for assessing disease activity. On the other hand, it can diminish the utility of anti-DNA testing in early disease screening, because important responses might only appear as the disease manifests clinically during the transition from pre-autoimmunity to autoimmunity.

Although the settings of clinical trials and routine care are quite distinct, the testing for anti-DNA antibodies, like that for ANAs, can be problematic for different reasons. ANA screening detects a wide variety of different autoantibody responses and involves many potential target antigens. By contrast, anti-DNA testing is narrow in its focus on a single target antigen; as such, it is amenable to the development of sensitive and specific analytical approaches that optimize antibody detection, and can be adapted for measuring only certain antibody subtypes (such as high-affinity subtypes). Substantial differences exist in qualitative and quantitative results regarding the detection of anti-DNA antibodies among the different assay kits currently available[81–86]. These differences can result from the molecular properties of particular DNA antigens used (such as circular DNA), as well as the experimental conditions of the assay. In fact, DNA is structurally heterogeneous because of sequence variability as well as base modification.

The anti-DNA antibodies present in patients with SLE can differ in their binding to different antigenic forms of DNA such as single-stranded and double-stranded DNA, as well as avidity. Because of these differences, some assays might fail to detect specific anti-DNA antibodies present in serum from patients. Indeed, many studies have demonstrated that the detection of anti-DNA antibodies is variable among commonly used techniques such as ELISA, Farr assay and *Crithidia luciliae* immunofluorescence assay[82]. Thus, a patient can be positive for anti-DNA antibodies according to one assay and negative according to another, although longitudinal studies indicate that, over time, a patient will express anti-DNA antibodies with similar immunochemical properties[81,84]. Not surprisingly, this situation can add to the uncertainty and confusion about the use of anti-DNA antibodies as biomarkers for any purpose. At present, the nature of the anti-DNA antibodies responsible for disease manifestations is not clear, and an assay with high specificity might have less utility for assessing disease activity than one with high sensitivity. This aspect of anti-DNA testing could be relevant in determining the need for new therapies or eligibility for clinical trials.

Using the results of ANA and anti-DNA antibody testing as entry criteria for clinical trials of SLE is tantamount to considering, under the same rubric, two serological biomarkers that are vastly different in clinical and immunological properties. Therefore, future studies are needed to determine which of the available assay formats is most informative and reliable, in order to transform these assays into theranostic markers or even companion diagnostics.

Box 1 | **Ways to enhance ANA testing**

The performance of current antinuclear antibody (ANA) assays, especially those based on immunofluorescence assays, suggests caution in their use as a general screen in settings of low pre-test probability. Given that most individuals who are positive for ANAs do not have disease, they can be considered as 'false positives'. Furthermore, the more frequently ANA testing occurs, the greater the number of false positive results will be. This situation can lead to more complicated and costly diagnostic testing as well as patient concerns. Pending the improved standardization of ANA assays, the interpretation of results will require more in-depth knowledge of the individual assays used, whether in the clinical or research setting. At a minimum, this effort requires knowledge of the particular assay format or kit used as well as the frequency of positivity in the general population. In settings where the suspicion of a diagnosis is high, health care providers should have access to alternative assays, especially when testing produces an unexpected negative result. Among the different types of ANA testing, multiplex assays have considerable popularity, particularly among clinical laboratories. Therefore, clinicians should understand the principles of these assays and work with clinical laboratories to develop strategies for utilizing multiplex and immunofluorescence assays in the most efficient and informative way. These two approaches can be used sequentially, and the decision of which assay to perform first should be based on the clinical context. Multiplex assays can ably detect some of the common disease-specific ANAs, but the number of different ANA specificites is large and includes specificities that are rare, even in the disease population. In this regard, there can be discrepancies between multiplex and immunofluorescence assays probably related to the sensitivity of these assays as well as the nature of the conformation of certain molecules, which can affect their antigenicity. Therefore, clinicians ordering these tests should understand that ANA testing is complicated, and that ANAs are not simple analytes. For both multiplex assays and ELISAs specific for a single response (such as anti-Sm antibodies), the results can be reported in a quantitative way just as results are reported for anti-DNA. In general, results of testing of antibodies to proteins such as Sm are given as either 'positive' or 'negative'. Given that many current therapies, especially those for SLE, aim to reduce B cell activation and the expression levels of ANAs, the assessment of their effects on a variety of specific ANA responses can be a valuable biomarker approach.

Conclusions

The ANA test is one of the most unusual in medicine because a positive result represents a classification criterion for the diagnosis of autoimmune disease, while at the same time ANA positivity is present in a substantial proportion of the healthy population. The basis for widespread ANA positivity is unknown; it might represent a predisposition to autoimmunity that is common among humans, especially women. Alternatively, positive results might reflect particular properties of the assays, which allow detection of many antibodies that do not reflect serious immune disturbances and lack relevance for immunopathogenesis. This situation complicates the use of ANA testing for diagnosis, especially when pre-test probability is low; it also limits the use of ANAs as biomarkers for pre-autoimmunity screening as well as to assess eligibility for clinical trials and treatment with certain therapeutic agents. By contrast, assays directed to particular autoantigens such as DNA provide more specific biomarkers (BOX 1).

Despite its shortcomings, ANA testing will remain commonly performed in the clinical setting to evaluate patients with rheumatic or musculoskeletal complaints. Certainly, ANA testing can be made more effective by recognizing the performance

characteristics of the assays used, the pre-testing probability of a disease and the demographic and clinical features of different ANA-associated diseases[27]. Importantly, health care providers who utilize this type of testing should be aware of its technical issues and recognize that different ANA assay kits might not be equivalent in providing biomarker information. Future assays that utilize a larger panel of autoantigens might, it is hoped, provide useful information in the clinical setting and the emerging interest in determining subsets of SLE for clinical trials and treatment. Such assays can also advance efforts at personalized medicine based on the operation of specific disease mechanisms that are revealed by the clinical and laboratory features of the individual patient.

David S. Pisetsky is at Medical Research Service, Durham Veterans Administration Medical Center, Box 151G, 508 Fulton Street, Durham, North Carolina 27705, USA; and at the Division of Rheumatology and Immunology, Duke University Medical Center, DUMC 3544, Durham, North Carolina 27710, USA.
david.pisetsky@duke.edu

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Competing interests statement
The author declares no competing interests.

OPINION

Biologics registers in RA: methodological aspects, current role and future applications

Elena Nikiphorou, Maya H. Buch and Kimme L. Hyrich

Abstract | The beginning of the 21st century saw a biopharmaceutical revolution in the treatment of inflammatory rheumatic diseases, particularly rheumatoid arthritis. The fast-evolving use of biologic therapies highlighted the need to develop registers at national and international levels with the aim of collecting long-term data on patient outcomes. Over the past 15 years, many biologics registers have contributed a wealth of data and provided robust and reliable evidence on the use, effectiveness and safety of these therapies. The unavoidable challenges posed by the continuous introduction of new therapies, particularly with regard to understanding their long-term safety, highlights the importance of learning from experience with established biologic therapies. In this Perspectives article, the role of biologics registers in bridging the evidence gap between efficacy in clinical trials and real-world effectiveness is discussed, with a focus on methodological aspects of registers, their unique features and challenges and their role going forward.

The discovery and introduction of therapy with biologic DMARDs, or 'biologics', represents one of the most crucial advances in the field of rheumatology. For patients with rheumatoid arthritis (RA), biologics have transformed what was, for many, an incurable and devastating disease into a disease that can be fully controlled[1]. Although multiple randomized clinical trials (RCTs) have demonstrated the efficacy of these therapies, the nature of RCT recruitment and short follow-up periods mean that efficacy (how a therapy performs under clinical trial conditions) might not directly translate into effectiveness (how a therapy performs under standard clinical practice conditions). A number of biologics registers have therefore been established within the rheumatology community so that additional data from 'real-world' practice can be captured and this evidence gap bridged. In this Perspectives article, we discuss the unique features, differences in methodological approaches and challenges in both the capture and the analysis of observational drug data when addressing questions around drug usage and effects in populations. We also highlight key lessons learnt by drawing examples from European registers during our discussion of potential future applications.

What is a biologics register?

The field of rheumatology has a long tradition of observational research[2]. With the advent of biologic therapies for RA, many existing observational patient registers adapted their data collection to focus on outcomes following the exposure of patients to biologic therapies. Several countries also established new national biologics registers with the primary goal of studying treatment outcomes following the use of biologics (FIG. 1).

In essence, a biologics register is an observational cohort study that captures detailed data on the exposure of patients to biologic therapies, such as details of underlying diagnoses and start and stop dates for therapies, as well as treatment outcomes. These outcomes might include disease activity, patient reported outcome measures (such as the Health Assessment Questionnaire) or the occurrence of new comorbidities or adverse events. Although the majority of registers capture data across all these areas, each register differs in design. For example, the UK[3] and Germany[4] established bespoke new cohort studies that recruited patients at the point of starting their first biologic therapy. Both registers also aimed to recruit a cohort of patients receiving conventional synthetic DMARDs (csDMARDs) as comparator groups. In the UK, the British Society for Rheumatology Biologics Register for RA (BSRBR-RA) was designed to fulfil set recruitment targets and then stop recruiting when these targets were reached, rather than to capture data from all patients receiving biologic therapy[3]. This design differs from that of registers based in countries that adapted or developed existing patient registers (such as Sweden, Denmark and Switzerland). In the Antirheumatic Therapies in Sweden (ARTIS) register[5], Danish National Registry for Biologic Therapy (DANBIO)[6] and Swiss Clinical Quality Management Programme for RA (SCQM-RA)[7], captured biologics data is embedded within a larger national patient register that aims to capture outcome data on all patients with RA, regardless of whether they receive biologic therapies or not. Both approaches have their strengths and weaknesses and provide valuable sources of data on the effects of biologic therapies. Bespoke biologics registers have the advantage of deep data capture, particularly surrounding the occurrence and details of adverse events. However, due to the increased workload of capturing such data, these registers might not always capture data comprehensively. Conversely, external data sources, such as those available in Scandinavia, represent a unique opportunity to optimize data quality and reduce bias (for example, by using hospitalization data collected independently of the rheumatology register).

The large sample sizes, long follow-up periods and real-life populations included in biologics registers provide a contrast to

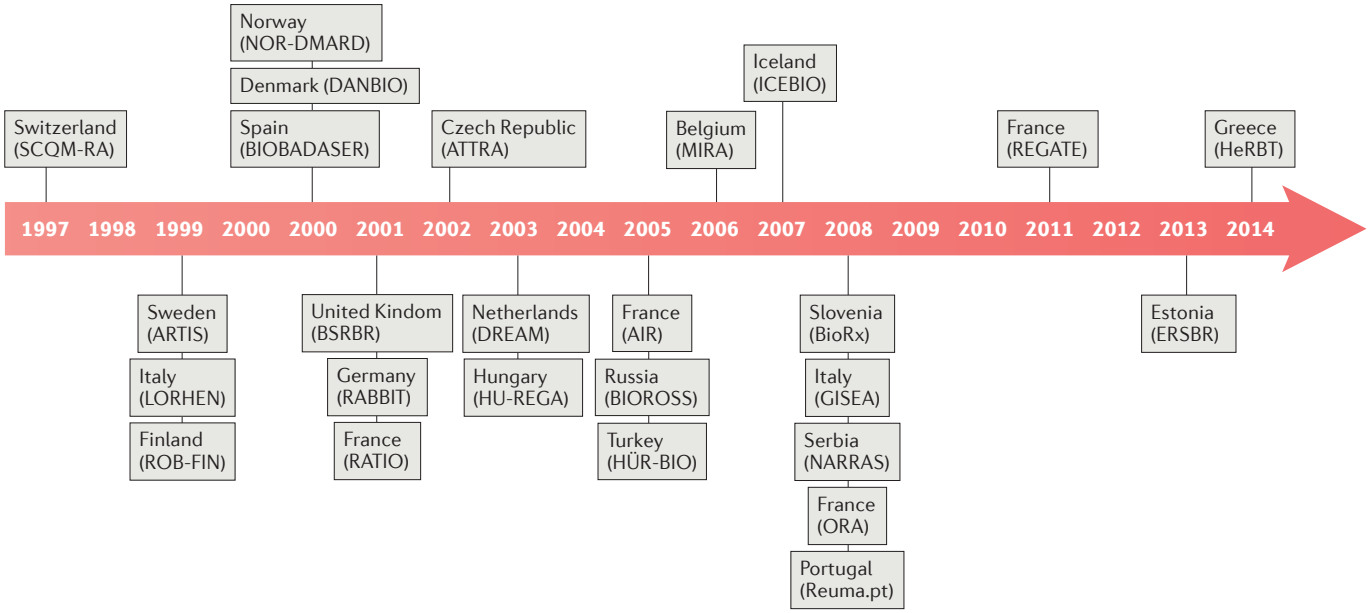

Figure 1 | **Timeline showing the founding of European biologics registers for rheumatoid arthritis.** Registers are listed alphabetically. AIR, Autoimmunity and Rituximab; ARTIS, Antirheumatic Therapies in Sweden; ATTRA, Czech National Registry of Patients Treated with Anti-TNF Drugs; BIOBADASER, Base de Datos de Productos Biológicos de la Sociedad Española de Reumatología; BIOROSS, Russian National Biologic Registry; BioRx, Slovenian National Biologic Registry; BSRBR, British Society for Rheumatology Biologics Register; DANBIO, Danish National Registry for Biologic Therapy; DREAM, Dutch Rheumatoid Arthritis Monitoring Registry; ERSBR, Estonian Society for Rheumatology Biologics Register; GISEA, Grupo Italiano di Studio Sulla Early Arthritis; HeRBT, Hellenic Registry for Biologic Therapies; HU-REGA, Hungarian National Biologic Registry; HÜR-BIO, Hacettepe University Rheumatology Biologic Registry; ICEBIO, Iceland National Biologics Registry; LORHEN, Lombardy Rheumatology Network; MIRA, MabThera in Rheumatoid Arthritis; NARRAS, National Registry of Patients with Rheumatoid Arthritis; NOR-DMARD, Norwegian Disease-Modifying Antirheumatic Drug Registry; ORA, Orencia in Rheumatoid Arthritis; RABBIT, Rheumatoid Arthritis Observation of Biologic Therapy; RATIO, Research Axed on Tolerance of Biotherapies; REGATE, Longitudinal Study on Patients with Rheumatoid Arthritis and Study on Tolerance and Efficacy of Tocilizumab (also known as REGistry-RoAcTEmra); Reuma.pt, The Rheumatic Diseases Portuguese Register; ROB-FIN, National Register of Biologic Treatment in Finland; SCQM-RA, Swiss Clinical Quality Management Programme for Rheumatoid Arthritis.

the relatively small and select homogeneous populations in RCTs, enabling improved external validation. Many registers also have links to national death databases and biorepositories, or have access to laboratory data, making them particularly suited for use in answering specific research questions[8]. The purpose, design and unique features of a selection of established RA biologics registers are outlined in TABLE 1.

Lessons from biologics registers

RCTs remain the benchmark for measuring the efficacy of new therapies; however, trials use stringent selection criteria, usually in an attempt to recruit a homogenous group of patients, which does not always represent the patients who will eventually receive the therapies being tested[9,10]. Clinical trials are not usually sufficiently powered to study the risk of less common outcomes (such as serious infection), and as recruitment and follow-up often occur over a short period of time, latent effects (such as the risk of malignancy) might not be observed. Additionally, RCTs provide no information on how clinical practice evolves over time. It is in these areas, therefore, that data from registers can complement data from clinical trials.

Biologics in clinical practice. Early reports from the German Rheumatoid Arthritis Observation of Biologic Therapy (RABBIT)[9] and Dutch Rheumatoid Arthritis Monitoring (DREAM)[10] registers showed that the majority of patients with RA receiving TNF inhibitors would not have been deemed eligible to participate in clinical trials. A proportion of these patients were too ill or disabled to participate, but there was also a proportion of patients whose disease was not active enough for them to be eligible. Data from the BSRBR-RA published in 2017 showed that patients with RA who started rituximab or tocilizumab as a first-line biologic had higher frequencies of important comorbidities (such as cancer or interstitial lung disease, conditions which often preclude participation in RCTs) than patients who started an anti-TNF agent as first-line therapy[11]. Another study, this time from the Swiss SCQM-RA register, found that biologics were more frequently prescribed as monotherapy than in combination with csDMARDs to older patients with comorbidities, a lower BMI, a longer disease duration, higher disease activity and who had had a high number of previous biologics[7]. The study of register data over time has also provided insights into secular changes in the use of biologics; these therapies are now prescribed earlier in the course of treatment, following exposure to fewer csDMARDs and lower cumulative doses of glucocorticoids, to patients with milder disability than in previous years[12,13].

Effectiveness of biologic therapies. If patient populations receiving biologics in the clinic differ from those included in RCTs, it follows that the expected response rates to therapy in these patients could also differ. In general, initial treatment responses are similar in registers to those observed in clinical trials[6,14,15] but, using data from registers, researchers can go beyond treatment response and analyse long-term

Table 1 | **Examples of biologics registers for rheumatoid arthritis**

Registry	Country	Purpose	Design & unique features
BSRBR[3,58]	UK	Established by the BSR to monitor patients with rheumatic diseases who have been prescribed biologics and to evaluate the long-term toxicity of these agents in clinical practice	• A nationwide register, formed by an alliance between the BSR, the pharmaceutical industry and the University of Manchester, UK • Designed as a national prospective study with patient enrolment being an essential part of the prescribing process • The register includes recruitment and collection of data from a parallel comparison group of patients consisting of those with active RA treated with conventional synthetic DMARDs • Externally linked with national mortality and malignancy registers
ARTIS[5,48,56]	Sweden	Developed to provide data on patients treated with biologics following a request from the Swedish Medical Product Agency to rheumatologists	• A national register that is overseen by the Swedish Rheumatology Association and integrated into clinical practice • Allows for multiple control groups to be used and linkage to external registers • Includes data from two regional registers (SSATG and STURE)
RABBIT[52]	Germany	Developed to assess the long-term safety of biologics	A nationwide prospective cohort study with an internal control group of patients who have switched DMARD; after discontinuation of treatment with biologics, the patients contribute to a second control group
DANBIO[6,76]	Denmark	Developed to assess treatment effectiveness, adverse events and quality of life; the aim was to be clinically useful to rheumatologists during consultations and to improve quality of care	• A national quality register • Designed to capture operational clinical data as part of routine care • Includes patients with RA, psoriatic arthritis and ankylosing spondylitis who are followed longitudinally
NOR-DMARD[77,78]	Norway	Developed to assess the effectiveness and safety of DMARDs in inflammatory arthropathies	• A five-centre registry covering approximately one-third of the population in Norway • Includes all DMARD prescriptions to patients with inflammatory arthropathies, including patients with RA
SCQM-RA[7,79]	Switzerland	The aim of this register was to improve quality of care for patients with RA through examination of outcomes in individual patients	• Longitudinal population-based cohort of patients with RA, supported by the Swiss Society of Rheumatology • Recruitment is solely undertaken by rheumatologists • Patients included in SCQM-RA have more severe disease and receive more biologic agents compared with patients with RA in the general population

ARTIS, Antirheumatic Therapies in Sweden; BSR, British Society for Rheumatology; BSRBR, British Society for Rheumatology Biologics Register; DANBIO, Danish National Registry for Biologic Therapy; NOR-DMARD, Norwegian Disease-Modifying Antirheumatic Drug Registry; RA, rheumatoid arthritis; RABBIT, Rheumatoid Arthritis Observation of Biologic Therapy; SCQM-RA, Swiss Clinical Quality Management Programme for Rheumatoid Arthritis; SSATG, South Swedish Arthritis Treatment Group; STURE, Stockholm TNFα Follow-up Registry.

treatment persistence[4,16,17], an area that cannot be easily explored in clinical trials. According to data from the BSRBR, 50% of patients, on average, have discontinued their first biologic by 5 years, for reasons of either ineffectiveness or adverse events[16].

Register data can also be used to compare different biologic therapies. Data from the Danish DANBIO[6] and the Italian Grupo Italiano di Studio sulla Early Arthritis (GISEA)[18] registers suggest that infliximab was associated with the lowest rates of treatment response, disease remission and drug survival; the highest rates of treatment response and disease remission were observed with adalimumab and the longest drug survival rates with etanercept[6]. The lack of head-to-head trials of the best second-line treatments for RA also directed focus towards register data for the comparison of outcomes among patients switching between different treatment options. The majority of evidence from register data, including the Spanish Base de Datos de Productos Biológicos de la Sociedad Española de Reumatología (BIOBADASER)[19] and Swedish Stockholm TNFα Follow-up Registry (STURE)[20], suggest that overall response rates are lower and drug-retention rates decrease in patients who switch to a second TNF inhibitor. Response to a second anti-TNF agent can differ according to the reason for failure of the first[21]. Data from the BSRBR[22] and SCQM-RA[23] registers reveal treatment with rituximab to be more effective than switching to an alternative anti-TNF agent in patients with RA who have persistently active disease despite treatment with a first anti-TNF agent, a finding supported by a non-register observational study[24] as well as by a large RCT[25]. These observations have formed a strong evidence base for decision-making in routine clinical practice. Although the majority of data collected to date has focused on TNF inhibitors, biologics registers are already providing information on the use of newer biologics, such as rituximab, abatacept and tocilizumab[26–30]. Data on these newer biologics is either incorporated into existing registers or collected in newly developed registers.

In addition to providing data for describing and comparing biologic treatment responses, registers have also produced data that can help to describe the type of patients who achieve a good response with anti-TNF therapy. Factors identified as being associated with a good response to treatment include young age[6], short disease duration[13], good functional status at the start of therapy[6,31,32] and never smoking[31,33–36]. Furthermore, where studied, most registers have confirmed improved treatment responses among patients who start anti-TNF therapy alongside methotrexate, even in the setting of previous methotrexate failure[14,16,32,37]. However, in all these examples, register data have shown that clinical data alone are not sufficient to predict which patients will have a good response to therapy, which has led to further biomarker studies in RA[38].

Safety of biologic therapies. The very large sample sizes and long follow-up periods of biologics registers have enabled an analysis of risk that goes beyond that available from clinical trials. Most registers have confirmed a small but statistically significant increase in the risk of serious infections occurring early in the course of anti-TNF therapy, which seems to decrease over time[26,39–43]. Further exploration of the data held within the German RABBIT register suggests that this observation is attributable both to a reduction in the number of patients at high risk of infection in the cohort, and to improvements in disease activity and reductions in steroid use among those patients who respond to therapy, thus reducing their overall infection risk[44]. Additionally, observational drug registers have enabled the study of the potential benefits of treatment with respect to safety outcomes, for example the association between use of anti-TNF agents and a reduced risk of cardiovascular events in patients with RA[45]. A number of registers have also published data on the observed risk of cancer in patients receiving biologics compared with patients receiving csDMARDs, and have not confirmed an increased risk of solid organ cancer or lymphoma[46–57] (see Supplementary information S1–S3 (tables)). Furthermore, biologics registers have enabled the study of outcomes in populations excluded from trials, such as patients with a history of cancer[52,58] and the elderly[43], and have revealed information about the risk of exposure to TNF inhibitors and other biologics during pregnancy[59,60]. The provision of further insights into the real-world safety of biologic therapies represents one of the most valuable aspects of register data.

Methodological challenges

Developing and running a biologics register requires thorough logistical and methodological planning to ensure completeness of data recording and adequate administrative support. In the following sections, we address some of the more common methodological and analytical challenges presented by biologics registers, as summarized in FIG. 2.

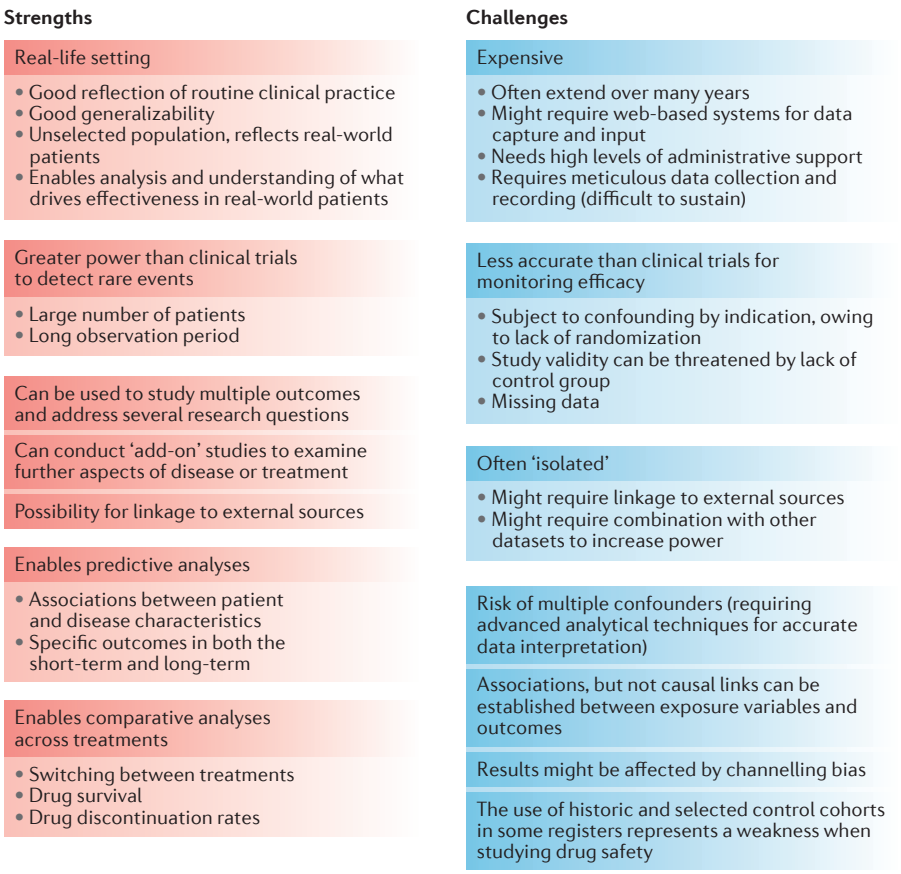

Figure 2 | **Strengths and challenges of biologics registers.** Using data from biologics registers confers specific benefits, but also has some drawbacks.

Patient recruitment and missing data. Recruitment into a register can be active or passive. Active recruitment presents more challenges to the clinician, as it involves an additional step, which, when added to the environment of a busy clinic, means that not all of the patients who are eligible for the register might be recruited. To ensure the successful development, maintenance and consistent contribution to a register, it is important to have motivated physicians with a genuine interest in and belief in the value of clinical data collection for research. Often, such contribution is voluntary; however, in some countries it is a mandatory duty for clinicians to contribute a minimum amount of data (usually pre-specified on paper or electronic forms) to biologics registers. Such data include details of patient demographics and information about therapies, including any adverse events and reasons for discontinuation. However, in busy clinical settings, accurately completing even the minimum amount of information requested can pose a challenge, leading to incomplete forms being submitted and further adding to the administrative workload of the register. In this respect, site reimbursement for recruiting patients into a register might provide an incentive to clinicians. Passive recruitment is, in theory, simpler; however, a potential challenge is the disconnection between the person reporting the data and the person recording it as to why the data need to be captured. This, in turn, could risk incomplete or missing data, which are likely to be a mixture of missing covariate data and missing outcomes.

Actively encouraging those involved with registers to report the proportion of missing data, especially when studying key outcomes, is necessary and could prompt more complete data collection. Reducing the amount of missing data and improving the accuracy of the data collected is important for the quality of analyses and, consequently, for the findings and conclusions made. To improve data accuracy, adequate administrative input and encouragement of physicians or collectors are crucial.

Data collection and input. Securing reliable long-term funding to ensure register sustainability and having a robust, high quality and, ideally, web-based platform for data input, access and extraction represent important challenges for registers. The depth of data collected depends on the type of register and its design, which is often dictated by the research question(s) being

studied. For example, some registers will collect data on the characteristics of patients and the disease, as well as treatment data and potential confounders. The actual process of data collection will depend on whether outcomes are reported or captured independently of the prescribing physician, or both.

Many registers use data linkage as a useful way of enriching source data. Data linkage enables further validation of events reported in source data, and ensures a more complete dataset, depending on the source of the linked data. Data linkage is particularly valuable when the linked data are in a mandatory national dataset, such as a national death or cancer register. The ability to validate the events captured through a linked route will depend upon the data capture methods of the independent data source.

Analytical challenges

Lack of randomization. The lack of randomization in the allocation of patients to treatments in routine clinical practice leads to confounding by indication, whereby observed outcomes might be related to the indication itself rather than to any exposure to the therapy. This lack of randomization, along with the absence of a control group and channelling bias, necessitate appropriate and often advanced statistical methodology when analysing data, such as the use of propensity scores, econometric selection models or other approaches[61]. It should be acknowledged, though, that even with the use of advanced statistical methods, these biases might not be fully overcome. Confounding by indication often stems from the clinical reasons driving treatment choice as a result of physician and patient perceptions of disease severity, prognosis and treatment effect. However, other, 'extraneous' aspects, including socioeconomic factors, can also influence these decisions; countering these aspects requires appropriate epidemiological design and careful selection of control groups and analytical techniques[62].

Time delays. The delay between the entry of a drug into the market and the accumulation of sufficient outcome data for valid analyses into the drug's efficacy and safety needs to be considered. The analytical challenge in this situation relates both to the accumulated exposure of the patient to the drug and to the latency of adverse events relating to the drug. The issues of incomplete or missing data, missing patients (lost to follow-up) and the power of the study to detect rare events need to be carefully considered, as even the largest national registers might not be sufficiently powered to measure the risk of very rare events, such as certain types of cancer. The insufficient power of individual registers to measure rare events, in particular, highlights one of the major benefits of using combined register data[47].

Pooled versus parallel analysis. Possible solutions to the issue of weakly powered individual registers are data pooling and parallel analysis with meta-analysis. A considered approach is necessary when using this type of analytical technique to account for differences in register design and types of data collected, as well as differences in health care systems and geographical and population differences. Aside from inherent variations in the characteristics of patients (such as different genetic backgrounds), the presence of endemic diseases (such as tuberculosis or HIV), comorbid conditions and differences in access to biologics can affect disease severity at the onset of treatment and therefore the response of a patient to treatment. These variations pose challenges when studying drug safety or effectiveness. One way to address these variations is to take into account any potential register-caused modification of effect by using multi-level, stratified analyses with data from individual patients. If analysis of individual patient data is not feasible, meta-analysis of register data is often the next-preferred option. Beyond the type and nature of data collected across registers, ethical restrictions and patient consent might also be important obstacles to data sharing and pooled analysis. The recognition of these issues has resulted in the publication by EULAR of a series of points to consider when designing and establishing a biologics register[63] (BOX 1).

Differences in recruitment patterns, data collected (items and definitions) and biologics prescription across registers is an important issue when pooling data for analysis[64]. To address these issues, the EULAR Study Group for Registers and Observational Studies (RODS)[64] specifically set out to compare patients starting treatment with biologics across Europe. This study, which involved 14 European biologics registers, acknowledged that differences in disease severity at the start of therapy do exist between countries, but also highlighted the lack of a common data model across Europe and the need for further work to harmonize data collection across registers[64]. Identifying a minimum core set of items for collection is therefore deemed to be

Box 1 | **Points to consider when establishing biologics registers**

General considerations
Examples of general considerations include defining the scientific questions that the register hopes to answer and considering the sample sizes and whether and what length of follow-up are needed.

Target population
A biologics register should define the eligibility criteria for the population to be included in the register.

Data items to be collected pertaining to the treatment and the treated condition
Identifying a minimum core set of variables to be collected is important in ensuring data completeness and comparability across studies.

Data items to be collected pertaining to outcomes
Collection of data items pertaining to outcomes should be undertaken in a complete, robust and transparent manner.

Follow-up methods
A register should ensure similar follow-up methods are applied to the treatment-exposed and comparison cohorts.

Data collection process and data collectors
The process of data collection should be defined, achieving clarity on who will be providing and entering data, but also defining and testing data capture and entry.

Ethical and legal considerations of data handling and storage
It is important to ensure the security of patient-identifiable information and compliance with local legislation in relation to data handling and storage.

Information for this Box was obtained from Dixon, W. G. *et al.* EULAR points to consider when establishing, analysing and reporting safety data of biologics registers in rheumatology. *Ann. Rheum. Dis.* **69**, 1596–1602 (2010).

useful in providing a common platform for data analysis across multiple registers. This premise forms the backbone of the EULAR Task Force on recommendations for the standardized content and structure of core data to facilitate patient care and observational research in RA.

The ability to standardize data collection across registers can lead to a better understanding of the reasons for heterogeneity in the results and conclusions between registers[8], as well as improving the interpretation and comparison of drug class-specific and drug-specific risks[65]. Existing initiatives involving pooled data analysis[27,29,30] have provided insights into the influence of characteristics intrinsic to patients (such as age) and to the disease (such as antibody status), and to extrinsic factors (such as geographical influences and variations in treatment practice). The growing interest in pooling datasets for common data analysis represents a potential future application of biologics registers that would both increase their power and provide information on a diverse population of patients[64,66].

How to best use register data

Many of the challenges and limitations discussed above will inevitably be present in any register, but this situation is acceptable as long as there is transparency as to the methodology and the limitations of the analysis[63]. Even discrepant findings can provide important information if the study design, analysis and data reporting are given careful consideration[8,67]. The emphasis of the research questions and outcomes being examined in biologics registers is changing over time, shifting from focusing on disease behaviour, improving disease activity and decreasing disability to focus on treatment effectiveness in different diseases[68] and on individualizing treatment. For example, the question of which biologic to choose after a patient experiences an inadequate response or an adverse event with a TNF inhibitor[69] is now a common research agenda. Although the majority of biologics registers initially recruited only patients with RA, registers have been extended over time to include data on use of biologics in patients with other conditions (for example, ankylosing spondylitis or psoriatic arthritis), enabling the study of important outcomes in these disease areas[70,71]. The knowledge gained from biologics registers over the past 15 years also provides a firm foundation for embarking on the collection of data for biosimilars, new classes of biologics and other advanced therapies. Several national rheumatology societies have already produced position papers on the use of biosimilars, recommending the registration of patients being treated with biosimilars into registers for surveillance of efficacy, safety and immunogenicity, following the strategy already ongoing for original biologics[72–74].

Bridging the effectiveness–efficacy gap. Reducing the discrepancies between effectiveness (in real-world conditions) and efficacy (in ideal circumstances) when evaluating new treatments would maximize the usefulness of information gathered by registers. Clinical trial data is useful for understanding efficacy of a therapy without the effect of confounding factors; however, efficacy across trial populations might not translate into equal effectiveness in patients in a real-world setting. With comparative effectiveness research becoming increasingly important, data from clinical trials are unlikely to provide answers to many important questions, in contrast to data from observational biologics registers[8]. Furthermore, biologics registers could be of value in studying subsets of the population that are not adequately studied in clinical trials, and in addressing the effectiveness, including the cost-effectiveness, of third-line and fourth-line biologics compared with the use of these drugs earlier in the treatment pathway.

Combined register–trial studies. With randomization being the only reliable method of controlling for confounding factors and enabling accurate comparison of treatment groups, clinical trials represent a strong foundation for evidence-based medicine[75]. However, running an adequately powered clinical trial incurs high costs, which, along with other limitations such as the select population of patients enrolled in RCTs (which might not represent an average patient seen in clinical practice), is an important problem. One possible solution would be to include a randomization module within a clinical register with unselected consecutive enrolment, thereby combining aspects of a prospective randomized trial with a large-scale clinical register[75]. Such an approach would potentially be an efficient and cost-effective future application of biologics registers, making it possible to obtain accurate answers to questions that data from clinical trials alone cannot provide.

Conclusions

Biologics have had a groundbreaking effect on the treatment of RA, yet the future of RA and its treatment is not solely dependent on these drugs. The intensified treatment regimens and treat-to-target approaches that have emerged along with new therapies necessitate high levels of vigilance and carefully conducted studies to assess the safety, efficacy and effectiveness of these new therapies. The establishment of several national biologics registers aimed at understanding real-world effectiveness and safety of therapies beyond that observed in RCTs fills an important gap in the literature, enhancing our understanding of real-life aspects of these therapies and their effect on disease progression and long-term outcomes. The rich repository of data within these registers will have an ongoing role in complementing clinical trial data. Although challenges remain, with advanced methodologies and new technologies on the horizon the potential for novel uses of biologics registers remains promising.

Elena Nikiphorou is at the Department of Academic Rheumatology, Weston Education Centre, Denmark Hill Campus, King's College London, London SE5 9RJ, UK; and at the Department of Rheumatology, Whittington Hospital, Magdala Avenue, London N19 5NF, UK.

Maya H. Buch is at the Leeds Institute of Rheumatic and Musculoskeletal Medicine, Chapel Allerton Hospital; and at the National Institute for Health Research (NIHR) Leeds Musculoskeletal Biomedical Research Unit, Chapel Allerton Hospital, Chapeltown Road, Leeds LS7 4SA, UK.

Kimme L. Hyrich is at the National Institute for Health Research (NIHR) Manchester Musculoskeletal Biomedical Research Unit, Manchester Academic Health Science Centre, Central Manchester University Hospitals NHS Foundation Trust; and at the Arthritis Research UK Centre for Epidemiology, Division of Musculoskeletal and Dermatological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK.

Correspondence to K.L.H.
kimme.hyrich@manchester.ac.uk

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Author contributions

All authors researched the data for the article, provided substantial contributions to discussions of its content, wrote the article and undertook review and/or editing of the manuscript before submission.

Competing interests statement

K.L.H. declares that she has received honoraria from Abbvie and Pfizer. M.H.B. declares that she has received grants from Pfizer and Roche and has been on the advisory board and/or provided lectures for Abbvie, Bristol-Myers Squibb, Pfizer, Roche and UCB. E.N. declares no competing interests.

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FURTHER INFORMATION

Recommendations for the standardised content and structure of core data to facilitate patient care and observational research in RA: http://eular.org/epidemiology_ongoing_initiatives.cfm

SUPPLEMENTARY INFORMATION

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